

## 5 MAMMALIAN RECEPTOR PROTEINS; RELATED REAGENTS AND METHODS

This application claims benefit of U.S. provisional Patent Application number 60/206,862, filed May 24, 2000.

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## FIELD OF THE INVENTION

The present invention relates to compositions and methods for affecting mammalian physiology, including immune system function. In particular, it provides methods to regulate development and/or the immune system. Diagnostic and therapeutic uses of these materials are also disclosed.

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## BACKGROUND OF THE INVENTION

Recombinant DNA technology refers generally to techniques of integrating genetic information from a donor source into vectors for subsequent processing, such as through introduction into a host, whereby the transferred genetic information is copied and/or expressed in the new environment. Commonly, the genetic information exists in the form of complementary DNA (cDNA) derived from messenger RNA (mRNA) coding for a desired protein product. The carrier is frequently a plasmid having the capacity to incorporate cDNA for later replication in a host and, in some cases, actually to control expression of the cDNA and thereby direct synthesis of the encoded product in the host. See, e.g., Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual, (2d ed.) vols. 1-3, CSH Press, NY.

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For some time, it has been known that the mammalian immune response is based on a series of complex cellular interactions, called the "immune network". Recent research has provided new insights into the inner workings of this network. While it remains clear that much of the immune response does, in fact, revolve around the network-like interactions of lymphocytes, macrophages, granulocytes, and other cells, immunologists now generally hold the opinion that soluble proteins, known as lymphokines, cytokines, or monokines, play critical roles in controlling these cellular interactions. Thus, there is considerable interest in the isolation, characterization, and mechanisms of action of cell modulatory factors, an understanding of which will lead to significant advancements in the diagnosis and therapy of numerous medical abnormalities, e.g., immune system disorders.

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The immune system of vertebrates consists of a number of organs and several different cell types. Two major cell types include the myeloid and lymphoid lineages. Among the lymphoid cell lineage are B cells, which were originally characterized as differentiating in fetal liver or adult bone marrow, and T cells, which were originally characterized as differentiating in the thymus. See, e.g., Paul (ed. 1998) Fundamental Immunology (4th ed.) Raven Press, New York; and Thomson (ed. 1994) The Cytokine Handbook 2d ed., Academic Press, San Diego. Lymphokines apparently mediate cellular activities in a variety of ways. They have been shown to support the proliferation, growth, and/or differentiation of cells, e.g., pluripotential hematopoietic stem cells, into vast numbers of progenitors comprising diverse cellular lineages which make up a complex immune system. Proper and balanced interactions between the cellular components are necessary for a healthy immune response. The different cellular lineages often respond in a different manner when lymphokines are administered in conjunction with other agents.

Cell lineages especially important to the immune response include two classes of lymphocytes: B-cells, which can produce and secrete immunoglobulins (proteins with the capability of recognizing and binding to foreign matter to effect its removal), and T-cells of various subsets that secrete lymphokines and induce or suppress the B-cells and various other cells (including other T-cells) making up the immune network. These lymphocytes interact with many other cell types.

Research to better understand and treat various immune disorders has been hampered by the general inability to maintain cells of the immune system in vitro. Immunologists have discovered that culturing many of these cells can be accomplished through the use of T-cell and other cell supernatants, which contain various growth factors, including many of the lymphokines.

Various growth and regulatory factors exist which modulate morphogenetic development. And many receptors for cytokines are also known. Often there are at least two critical subunits in the functional receptor. See, e.g., Gonda and D'Andrea (1997) Blood 89:355-369; Presky, et al. (1996) Proc. Nat'l Acad. Sci. USA 93:14002-14007; Drachman and Kaushansky (1995) Curr. Opin. Hematol. 2:22-28; Theze (1994) Eur. Cytokine Netw. 5:353-368; and Lemmon and Schlessinger (1994) Trends Biochem. Sci. 19:459-463.

From the foregoing, it is evident that the discovery and development of new soluble proteins and their receptors, including ones similar to lymphokines, should contribute to new therapies for a wide range of degenerative or abnormal conditions which directly or indirectly involve development, differentiation, or function, e.g., of the

immune system and/or hematopoietic cells. In particular, the discovery and understanding of novel receptors for lymphokine-like molecules which enhance or potentiate the beneficial activities of other lymphokines would be highly advantageous. However, the lack of understanding of how the immune system is regulated or differentiates has blocked the ability to advantageously modulate the normal defensive mechanisms to biological challenges. Medical conditions characterized by abnormal or inappropriate regulation of the development or physiology of relevant cells thus remain unmanageable. The discovery and characterization of specific cytokines and their receptors will contribute to the development of therapies for a broad range of degenerative or other conditions which affect the immune system, hematopoietic cells, as well as other cell types. The present invention provides new receptors for ligands exhibiting similarity to cytokine like compositions and related compounds, and methods for their use.

#### SUMMARY OF THE INVENTION

The present invention is directed to novel receptors related to cytokine receptors, e.g., primate, cytokine receptor like molecular structures, designated DNAX Cytokine Receptor Subunits (DCRS), and their biological activities. In particular, it provides description of various subunits, designated DCRS6, DCRS7, DCRS8, DCRS9, and DCRS10. Primate, e.g, human, and rodent, e.g., mouse, embodiments of the various subunits are provided. It includes nucleic acids coding for the polypeptides themselves and methods for their production and use. The nucleic acids of the invention are characterized, in part, by their homology to cloned complementary DNA (cDNA) sequences enclosed herein.

~~The present invention provides a composition of matter selected from: a substantially pure or recombinant polypeptide comprising at least three distinct nonoverlapping segments of at least four amino acids identical to segments of SEQ ID NO: 2, 5, 8, 11, 23, or 26; a substantially pure or recombinant polypeptide comprising at least three distinct nonoverlapping segments of at least four amino acids identical to segments of SEQ ID NO: 14; a substantially pure or recombinant polypeptide comprising at least two distinct nonoverlapping segments of at least five amino acids identical to segments of SEQ ID NO: 14; a natural sequence DCRS8 comprising mature SEQ ID NO: 14; a fusion polypeptide comprising DCRS8 sequence; a substantially pure or recombinant polypeptide comprising at least three distinct nonoverlapping segments of at least four amino acids identical to segments of SEQ ID NO: 17 or 20; a substantially pure or recombinant polypeptide comprising at least two distinct nonoverlapping segments of at least five amino acids identical to segments of SEQ ID NO: 17 or 20; a natural~~

sequence DCRS9 comprising mature SEQ ID NO: 17 or 20; or a fusion polypeptide comprising DCRS9 sequence. Preferably, wherein the distinct nonoverlapping segments of identity include: one of at least eight amino acids; one of at least four amino acids and a second of at least five amino acids; at least three segments of at least four, five, and six amino acids, or one of at least twelve amino acids. In other embodiments, the polypeptide: comprises a mature sequence of Tables 1, 2, 3, 4, or 5; is an unglycosylated form of DCRS8 or DCRS9; is from a primate, such as a human; comprises at least seventeen amino acids of SEQ ID NO: 14 or 17; exhibits at least four nonoverlapping segments of at least seven amino acids of SEQ ID NO: 14 or 17; is a natural allelic variant of DCRS8 or DCRS9; has a length at least about 30 amino acids; exhibits at least two non-overlapping epitopes which are specific for a primate DCRS8 or DCRS9; is glycosylated; has a molecular weight of at least 30 kD with natural glycosylation; is a synthetic polypeptide; is attached to a solid substrate; is conjugated to another chemical moiety; is a 5-fold or less substitution from natural sequence; or is a deletion or insertion variant from a natural sequence.

The invention further embraces a composition comprising: a substantially pure DCRS8 or DCRS9 and another cytokine receptor family member; a sterile DCRS8 or DCRS9 polypeptide; the DCRS8 or DCRS9 polypeptide and a carrier, wherein the carrier is: an aqueous compound, including water, saline, and/or buffer; and/or formulated for oral, rectal, nasal, topical, or parenteral administration. Additional embodiments include a polypeptide comprising: mature protein sequence of Tables 1, 2, 3, 4, or 5; a detection or purification tag, including a FLAG, His6, or Ig sequence; or sequence of another cytokine receptor protein. Kit embodiments include ones comprising a described polypeptide, and: a compartment comprising the protein or polypeptide; or instructions for use or disposal of reagents in the kit.

Binding compositions are provided, e.g., comprising an antigen binding site from an antibody, which specifically binds to a natural DCRS8 or DCRS9 polypeptide, wherein: the binding compound is in a container; the DCRS8 or DCRS9 polypeptide is from a human; the binding compound is an Fv, Fab, or Fab2 fragment; the binding compound is conjugated to another chemical moiety; or the antibody: is raised against a peptide sequence of a mature polypeptide of Table 3 or 4; is raised against a mature DCRS8 or DCRS9; is raised to a purified human DCRS8 or DCRS9; is immunoselected; is a polyclonal antibody; binds to a denatured DCRS8 or DCRS9; exhibits a K<sub>d</sub> to antigen of at least 30  $\mu$ M; is attached to a solid substrate, including a bead or plastic membrane; is in a sterile composition; or is detectably labeled, including a radioactive or fluorescent label. Kits include ones comprising such a binding compound, and: a compartment

comprising the binding compound; or instructions for use or disposal of reagents in the kit.

The invention also provides methods of producing an antigen:antibody complex, comprising contacting under appropriate conditions a primate DCRS8 or DCRS9 polypeptide with a described antibody, thereby allowing the complex to form. Preferred methods include ones wherein: the complex is purified from other cytokine receptors; the complex is purified from other antibody; the contacting is with a sample comprising an interferon; the contacting allows quantitative detection of the antigen; the contacting is with a sample comprising the antibody; or the contacting allows quantitative detection of the antibody. Further compositions include those comprising: a sterile binding compound, as described, or the binding compound and a carrier, wherein the carrier is: an aqueous compound, including water, saline, and/or buffer; and/or formulated for oral, rectal, nasal, topical, or parenteral administration.

~~Nucleic acid compositions include an isolated or recombinant nucleic acid encoding a described polypeptide wherein the: DCRS8 or DCRS9 is from a human; or the nucleic acid: encodes an antigenic peptide sequence of Table 3 or 4; encodes a plurality of antigenic peptide sequences of Table 3 or 4; exhibits identity over at least thirteen nucleotides to a natural cDNA encoding the segment; is an expression vector; further comprises an origin of replication; is from a natural source; comprises a detectable label; comprises synthetic nucleotide sequence; is less than 6 kb, preferably less than 3 kb; is from a primate; comprises a natural full length coding sequence; is a hybridization probe for a gene encoding the DCRS8 or DCRS9; or is a PCR primer, PCR product, or mutagenesis primer. Also provided are a cell or tissue comprising such a recombinant nucleic acid, e.g., where the cell is: a prokaryotic cell; a eukaryotic cell; a bacterial cell; a yeast cell; an insect cell; a mammalian cell; a mouse cell; a primate cell; or a human cell.~~

Kit embodiments include those comprising a described nucleic acid and: a compartment comprising the nucleic acid; a compartment further comprising a primate DCRS8 or DCRS9 polypeptide; or instructions for use or disposal of reagents in the kit.

~~Other nucleic acids provided include ones which: hybridize under wash conditions of 30 minutes at 30° C and less than 2M salt to the coding portion of SEQ ID NO: 13 or 16; or exhibit identity over a stretch of at least about 30 nucleotides to a primate DCRS8 or DCRS9. Preferably, such will be nucleic acids where: the wash conditions are: at 45° C and/or 500 mM salt; at 55° C and/or 150 mM salt; or the stretch is at least 55 or 75 nucleotides.~~

Also provided are methods of modulating physiology or development of a cell or tissue culture cells comprising contacting the cell with an agonist or antagonist of a

mammalian DCRS8 or DCRS9. Preferably, the cell is transformed with a nucleic acid encoding the DCRS8 or DCRS9 and another cytokine receptor subunit.

## DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

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### OUTLINE

#### I. General

#### II. Activities

#### III. Nucleic acids

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- A. encoding fragments, sequence, probes
- B. mutations, chimeras, fusions
- C. making nucleic acids
- D. vectors, cells comprising

#### IV. Proteins, Peptides

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- A. fragments, sequence, immunogens, antigens
- B. muteins
- C. agonists/antagonists, functional equivalents
- D. making proteins

#### V. Making nucleic acids, proteins

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- A. synthetic
- B. recombinant
- C. natural sources

#### VI. Antibodies

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- A. polyclonals
- B. monoclonal
- C. fragments; Kd
- D. anti-idiotypic antibodies
- E. hybridoma cell lines

#### VII. Kits and Methods to quantify DCRSs

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- A. ELISA
- B. assay mRNA encoding
- C. qualitative/quantitative
- D. kits

#### VIII. Therapeutic compositions, methods

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- A. combination compositions
- B. unit dose
- C. administration

#### IX. Screening

#### X. Ligands

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#### I. General

The present invention provides the amino acid sequence and DNA sequence of mammalian, herein primate, cytokine receptor-like subunit molecules, these designated DNAX Cytokine Receptor Subunits 6 (DCRS6), 7 (DCRS7), 8 (DCRS8), 9 (DCRS9), and 10 (DCRS10) having particular defined properties, both structural and biological.

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Various cDNAs encoding these molecules were obtained from primate, e.g., human, and/or rodent, e.g., mouse, cDNA sequence libraries. Other primate or other mammalian counterparts would also be desired.

Some of the standard methods applicable are described or referenced, e.g., in  
 5 Maniatis, et al. (1982) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor Press; Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual, (2d ed.), vols. 1-3, CSH Press, NY; Ausubel, et al., Biology, Greene Publishing Associates, Brooklyn, NY; or Ausubel, et al. (1987 and periodic supplements) Current Protocols in Molecular Biology, Greene/Wiley, New York; each of which is  
 10 incorporated herein by reference.

~~NSA6~~ <sup>146</sup> Nucleotide (SEQ ID NO: 1) and corresponding amino acid sequence (SEQ ID NO: 2) of a primate, e.g., human, DCRS6 coding segment is shown in Table 1 along with reverse translation (SEQ ID NO: 3). Rodent, e.g., mouse, counterpart sequences are provided, e.g., SEQ ID NO: 4-6.

~~NSA7~~ <sup>147</sup> Similarly, nucleotide (SEQ ID NO: 7) and corresponding amino acid sequence (SEQ ID NO: 8) of a primate, e.g., human, DCRS7 coding segment is shown in Table 2 along with reverse translation (SEQ ID NO: 9). Rodent, e.g., mouse, counterpart sequences are provided, e.g., SEQ ID NO: 10-12. Nucleotide (SEQ ID NO: 13) and corresponding amino acid sequence (SEQ ID NO: 14) of a primate, e.g., human, DCRS8 coding segment is shown in Table 3 along with reverse translation (SEQ ID NO: 15).

~~NSA8~~ <sup>148</sup> Nucleotide (SEQ ID NO: 16) and corresponding amino acid sequence (SEQ ID NO: 17) of a primate, e.g., human, DCRS9 coding segment is shown in Table 4 along with reverse translation (SEQ ID NO: 18). Rodent, e.g., mouse, counterpart sequences are provided, e.g., SEQ ID NO: 19-21. Nucleotide (SEQ ID NO: 22) and corresponding  
 25 amino acid sequence (SEQ ID NO: 23) of a primate, e.g., human, DCRS10 coding segment is shown in Table 5 along with reverse translation (SEQ ID NO: 24). Rodent, e.g., mouse, counterpart sequences are provided, e.g., SEQ ID NO: 26-27.

30 Table 1: Nucleotide and polypeptide sequences of DNAX Cytokine Receptor Subunit like embodiments (DCRS6). Primate, e.g., human, embodiment (see SEQ ID NO: 1 and 2). Predicted signal sequence indicated, but may vary by a few positions and depending upon cell type.

35	gcg atg tcg ctc gtg ctg cta agc ctg gcc gcg ctg tgc agg agc gcc	48
	Met Ser Leu Val Leu Leu Ser Leu Ala Ala Leu Cys Arg Ser Ala	
	-10 -5 -1 1	
40	gta ccc cga gag ccg acc gtt caa tgt ggc tct gaa act ggg cca tct	96
	Val Pro Arg Glu Pro Thr Val Gln Cys Gly Ser Glu Thr Gly Pro Ser	
	5 10 15	

	cca	gag	tgg	atg	cta	caa	cat	gat	cta	atc	ccg	gga	gac	ttg	agg	gac	144
	Pro	Glu	Trp	Met	Leu	Gln	His	Asp	Leu	Ile	Pro	Gly	Asp	Leu	Arg	Asp	
			20					25					30				
5	ctc	cga	gta	gaa	cct	gtt	aca	act	agt	gtt	gca	aca	ggg	gac	tat	tca	192
	Leu	Arg	Val	Glu	Pro	Val	Thr	Thr	Ser	Val	Ala	Thr	Gly	Asp	Tyr	Ser	
		35					40					45					
10	att	ttg	atg	aat	gta	agc	tgg	gta	ctc	cgg	gca	gat	gcc	agc	atc	cgc	240
	Ile	Leu	Met	Asn	Val	Ser	Trp	Val	Leu	Arg	Ala	Asp	Ala	Ser	Ile	Arg	
	50					55					60					65	
15	ttg	ttg	aag	gcc	acc	aag	att	tgt	gtg	acg	ggc	aaa	agc	aac	ttc	cag	288
	Leu	Leu	Lys	Ala	Thr	Lys	Ile	Cys	Val	Thr	Gly	Lys	Ser	Asn	Phe	Gln	
					70					75					80		
	tcc	tac	agc	tgt	gtg	agg	tgc	aat	tac	aca	gag	gcc	ttc	cag	act	cag	336
	Ser	Tyr	Ser	Cys	Val	Arg	Cys	Asn	Tyr	Thr	Glu	Ala	Phe	Gln	Thr	Gln	
20				85					90					95			
	acc	aga	ccc	tct	ggg	ggg	aaa	tgg	aca	ttt	tcc	tat	atc	ggc	ttc	cct	384
	Thr	Arg	Pro	Ser	Gly	Gly	Lys	Trp	Thr	Phe	Ser	Tyr	Ile	Gly	Phe	Pro	
			100					105					110				
25	gta	gag	ctg	aac	aca	gtc	tat	ttc	att	ggg	gcc	cat	aat	att	cct	aat	432
	Val	Glu	Leu	Asn	Thr	Val	Tyr	Phe	Ile	Gly	Ala	His	Asn	Ile	Pro	Asn	
		115					120					125					
30	gca	aat	atg	aat	gaa	gat	ggc	cct	tcc	atg	tct	gtg	aat	ttc	acc	tca	480
	Ala	Asn	Met	Asn	Glu	Asp	Gly	Pro	Ser	Met	Ser	Val	Asn	Phe	Thr	Ser	
	130					135					140					145	
35	cca	ggc	tgc	cta	gac	cac	ata	atg	aaa	tat	aaa	aaa	aag	tgt	gtc	aag	528
	Pro	Gly	Cys	Leu	Asp	His	Ile	Met	Lys	Tyr	Lys	Lys	Lys	Cys	Val	Lys	
				150						155					160		
	gcc	gga	agc	ctg	tgg	gat	ccg	aac	atc	act	gct	tgt	aag	aag	aat	gag	576
	Ala	Gly	Ser	Leu	Trp	Asp	Pro	Asn	Ile	Thr	Ala	Cys	Lys	Lys	Asn	Glu	
40				165					170					175			
	gag	aca	gta	gaa	gtg	aac	ttc	aca	acc	act	ccc	ctg	gga	aac	aga	tac	624
	Glu	Thr	Val	Glu	Val	Asn	Phe	Thr	Thr	Thr	Pro	Leu	Gly	Asn	Arg	Tyr	
			180					185					190				
45	atg	gct	ctt	atc	caa	cac	agc	act	atc	atc	ggg	ttt	tct	cag	gtg	ttt	672
	Met	Ala	Leu	Ile	Gln	His	Ser	Thr	Ile	Ile	Gly	Phe	Ser	Gln	Val	Phe	
		195					200					205					
50	gag	cca	cac	cag	aag	aaa	caa	acg	cga	gct	tca	gtg	gtg	att	cca	gtg	720
	Glu	Pro	His	Gln	Lys	Lys	Gln	Thr	Arg	Ala	Ser	Val	Val	Ile	Pro	Val	
	210					215					220					225	
55	act	ggg	gat	agt	gaa	ggg	gct	acg	gtg	cag	ctg	act	cca	tat	ttt	cct	768
	Thr	Gly	Asp	Ser	Glu	Gly	Ala	Thr	Val	Gln	Leu	Thr	Pro	Tyr	Phe	Pro	
					230					235					240		



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qayaayaaya arwsnaarcc nggnggntgg ytnccnytny tnytnytnws nytnytnngtn 900

gcnaentggg tnytngtngc nggnathtay ytnatgtggm gncaygarmg nathaaraar 960  
 5 acnwsnttyw snacnacnac nytnytnccn ccnathaarg tnytngtngt ntayccnwsn 1020  
 garathtgyt tycaycayac nathtgytay ttyacngart tyytncaraa ycaytgymgn 1080  
 wsngargtna thytnagaraa rtggcaraar aaraarathg cngaratggg nccngtncar 1140  
 10 tggtyngcna cncaraaraa rgcngcngay aargtngtnt tyytnytnws naaygaygtn 1200  
 aaywsngtnt gygayggnac ntgyggnaar wsngarggnw snccnwsnga raaywsncar 1260  
 15 gayytnttyc cnytngcntt yaayytntty tgywsngayy tnmgnwsnca rathcayytn 1320  
 cayaartayg tngtngtnta yttymngnar athgayacna argaygayta yaaygcnytn 1380  
 wsngtntgyc cnaartayca yytnatgaar gaygcnacng cnttytgygc ngarytnytn 1440  
 20 caygtnaarc arcargtnws ngcnggnaar mgnwsncarg cntgycaiga yggntgytgy 1500  
 wsnytn 1506

25 Rodent, e.g., mouse embodiment (see SEQ ID NO: 4 and 5).

gat ttc agc agc cag acg cat ctg cac aaa tac ctg gag gtc tat ctt 48  
 Asp Phe Ser Ser Gln Thr His Leu His Lys Tyr Leu Glu Val Tyr Leu  
 1 5 10 15  
 30 ggg gga gca gac ctc aaa ggc gac tat aat gcc ctg agt gtc tgc ccc 96  
 Gly Gly Ala Asp Leu Lys Gly Asp Tyr Asn Ala Leu Ser Val Cys Pro  
 20 25 30  
 35 caa tat cat ctc atg aag gac gcc aca gct ttc cac aca gaa ctt ctc 144  
 Gln Tyr His Leu Met Lys Asp Ala Thr Ala Phe His Thr Glu Leu Leu  
 35 40 45  
 40 aag gct acg cag agc atg tca gtg aag aaa cgc tca caa gcc tgc cat 192  
 Lys Ala Thr Gln Ser Met Ser Val Lys Lys Arg Ser Gln Ala Cys His  
 50 55 60  
 45 gat agc tgt tca ccc ttg tagtccaccc gggggaatag agactctgaa 240  
 Asp Ser Cys Ser Pro Leu  
 65 70  
 50 gccttcctac tctcccttcc agtgacaaat gctgtgtgac gactctgaaa tgtgtgggag 300  
 aggctgtgtg gaggtagtgc tatgtacaaa cttgctttaa aactggagtt tgcaaagtca 360  
 acctgagcat acacgcctga ggctagtcac tggctggatt tatgaagaca acacagttac 420  
 agacaataat gagtgggacc tacatttggg atatacccaa agctgggtaa tgattatcac 480  
 55 tgagaaccac gactctggc catgaggtaa tacggcactt ccctgtcagg ctgtctgtca 540  
 ggttgggtct gtcttgact gcccatgctc tatgtctcac gtagaccgtt ttgtaacatt 600  
 ttaatctgtt aatgaataat ccgtttggga ggctctc 637

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DFSSQTHLHKYLEVYLGGADLKGDNALSVCPQYHLMKDATAFHTELLKATQSMSVKKRSQACHDSCSPL.

5 Reverse translation of rodent, e.g., mouse, DCRS6 (SEQ ID NO: 6):

gayttywsnw sncaracnca yytncayaar tayytnngarg tntayytnngg nggngcngay 60  
 ytnaarggng aytayaaygc nytnwsngtn tgyccncart aycayytnat gaargaygcn 120  
 10 acngcnttyc ayacngaryt nytnaargcn acncarwsna tgwsngtnaa raarmgnwsn 180  
 cargcntgyc aygaywsntg ywsnccnytn 210

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Table 2: Nucleotide and polypeptide sequences of DNAX Cytokine Receptor Subunit like  
 embodiments (DCRS7). Primate, e.g., human, embodiment (see SEQ ID NO: 7 and 8).  
 Predicted signal sequence indicated, but may vary by a few positions and depending upon cell  
 type.

20

gagtcaggac tcccaggaca gagagtgcac aaactaccca gcacagcccc ctccgcccc 60

tctggaggct gaagagggat tccagcccct gccaccaca gacacgggct gactgggggtg 120

25

tctgcccccc ttggggggcan ccacagggcc tcaggcctgg gtgccacctg gcactagaag 180

atg cct gtg ccc tgg ttc ttg ctg tcc ttg gca ctg ggc cga agc cag 228  
 Met Pro Val Pro Trp Phe Leu Leu Ser Leu Ala Leu Gly Arg Ser Gln  
 -20 -15 -10 -5

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tgg atc ctt tct ctg gag agg ctt gtg ggg cct cag gac gct acc cac 276  
 Trp Ile Leu Ser Leu Glu Arg Leu Val Gly Pro Gln Asp Ala Thr His  
 -1 1 5 10

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tgc tct ccg ggc ctc tcc tgc cgc ctc tgg gac agt gac ata ctc tgc 324  
 Cys Ser Pro Gly Leu Ser Cys Arg Leu Trp Asp Ser Asp Ile Leu Cys  
 15 20 25

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ctg cct ggg gac atc gtg cct gct ccg ggc ccc gtg ctg gcg cct acg 372  
 Leu Pro Gly Asp Ile Val Pro Ala Pro Gly Pro Val Leu Ala Pro Thr  
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cac ctg cag aca gag ctg gtg ctg agg tgc cag aag gag acc gac tgt 420  
 His Leu Gln Thr Glu Leu Val Leu Arg Cys Gln Lys Glu Thr Asp Cys  
 45 50 55 60

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gac ctc tgt ctg cgt gtg gct gtc cac ttg gcc gtg cat ggg cac tgg 468  
 Asp Leu Cys Leu Arg Val Ala Val His Leu Ala Val His Gly His Trp  
 65 70 75

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gaa gag cct gaa gat gag gaa aag ttt gga gga gca gct gac tta ggg 516  
 Glu Glu Pro Glu Asp Glu Glu Lys Phe Gly Gly Ala Ala Asp Leu Gly  
 80 85 90

gtg gag gag cct agg aat gcc tct ctc cag gcc caa gtc gtg ctc tcc 564  
 Val Glu Glu Pro Arg Asn Ala Ser Leu Gln Ala Gln Val Val Leu Ser  
 95 100 105

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		ttc	cag	gcc	tac	cct	act	gcc	cgc	tgc	gtc	ctg	ctg	gag	gtg	caa	gtg	612
		Phe	Gln	Ala	Tyr	Pro	Thr	Ala	Arg	Cys	Val	Leu	Leu	Glu	Val	Gln	Val	
		110						115					120					
5		cct	gct	gcc	ctt	gtg	cag	ttt	ggg	cag	tct	gtg	ggc	tct	gtg	gta	tat	660
		Pro	Ala	Ala	Leu	Val	Gln	Phe	Gly	Gln	Ser	Val	Gly	Ser	Val	Val	Tyr	
		125					130					135					140	
10		gac	tgc	ttc	gag	gct	gcc	cta	ggg	agt	gag	gta	cga	atc	tgg	tcc	tat	708
		Asp	Cys	Phe	Glu	Ala	Ala	Leu	Gly	Ser	Glu	Val	Arg	Ile	Trp	Ser	Tyr	
					145						150					155		
15		act	cag	ccc	agg	tac	gag	aag	gaa	ctc	aac	cac	aca	cag	cag	ctg	cct	756
		Thr	Gln	Pro	Arg	Tyr	Glu	Lys	Glu	Leu	Asn	His	Thr	Gln	Gln	Leu	Pro	
					160					165					170			
		gac	tgc	agg	ggg	ctc	gaa	gtc	tgg	aac	agc	atc	ccg	agc	tgc	tgg	gcc	804
		Asp	Cys	Arg	Gly	Leu	Glu	Val	Trp	Asn	Ser	Ile	Pro	Ser	Cys	Trp	Ala	
				175					180					185				
20		ctg	ccc	tgg	ctc	aac	gtg	tca	gca	gat	ggg	gac	aac	gtg	cat	ctg	gtt	852
		Leu	Pro	Trp	Leu	Asn	Val	Ser	Ala	Asp	Gly	Asp	Asn	Val	His	Leu	Val	
			190					195					200					
25		ctg	aat	gtc	tct	gag	gag	cag	cac	ttc	ggc	ctc	tcc	ctg	tac	tgg	aat	900
		Leu	Asn	Val	Ser	Glu	Glu	Gln	His	Phe	Gly	Leu	Ser	Leu	Tyr	Trp	Asn	
		205				210					215					220		
30		cag	gtc	cag	ggc	ccc	cca	aaa	ccc	cgg	tgg	cac	aaa	aac	ctg	act	gga	948
		Gln	Val	Gln	Gly	Pro	Pro	Lys	Pro	Arg	Trp	His	Lys	Asn	Leu	Thr	Gly	
					225					230					235			
35		ccg	cag	atc	att	acc	ttg	aac	cac	aca	gac	ctg	gtt	ccc	tgc	ctc	tgt	996
		Pro	Gln	Ile	Ile	Thr	Leu	Asn	His	Thr	Asp	Leu	Val	Pro	Cys	Leu	Cys	
					240				245					250				
40		att	cag	gtg	tgg	cct	ctg	gaa	cct	gac	tcc	gtt	agg	acg	aac	atc	tgc	1044
		Ile	Gln	Val	Trp	Pro	Leu	Glu	Pro	Asp	Ser	Val	Arg	Thr	Asn	Ile	Cys	
				255				260					265					
45		ccc	ttc	agg	gag	gac	ccc	cgc	gca	cac	cag	aac	ctc	tgg	caa	gcc	gcc	1092
		Pro	Phe	Arg	Glu	Asp	Pro	Arg	Ala	His	Gln	Asn	Leu	Trp	Gln	Ala	Ala	
			270					275					280					
50		cga	ctg	cga	ctg	ctg	acc	ctg	cag	agc	tgg	ctg	ctg	gac	gca	ccg	tgc	1140
		Arg	Leu	Arg	Leu	Leu	Thr	Leu	Gln	Ser	Trp	Leu	Leu	Asp	Ala	Pro	Cys	
			285				290				295					300		
55		tgc	ctg	ccc	gca	gaa	gcg	gca	ctg	tgc	tgg	cgg	gct	ccg	ggg	ggg	gac	1188
		Ser	Leu	Pro	Ala	Glu	Ala	Ala	Leu	Cys	Trp	Arg	Ala	Pro	Gly	Gly	Asp	
				305						310					315			
60		ccc	tgc	cag	cca	ctg	gtc	cca	ccg	ctt	tcc	tgg	gag	aat	gtc	act	gtg	1236
		Pro	Cys	Gln	Pro	Leu	Val	Pro	Pro	Leu	Ser	Trp	Glu	Asn	Val	Thr	Val	
				320					325						330			
65		gac	gtg	aac	agc	tgc	gag	aag	ctg	cag	ctg	cag	gag	tgc	ttg	tgg	gct	1284
		Asp	Val	Asn	Ser	Ser	Glu	Lys	Leu	Gln	Leu	Gln	Glu	Cys	Leu	Trp	Ala	
				335				340					345					

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gac tcc ctg ggg cct ctc aaa gac gat gtg cta ctg ttg gag aca cga	1332
Asp Ser Leu Gly Pro Leu Lys Asp Asp Val Leu Leu Leu Glu Thr Arg	
350 355 360	
ggc ccc cag gac aac aga tcc ctc tgt gcc ttg gaa ccc agt ggc tgt	1380
Gly Pro Gln Asp Asn Arg Ser Leu Cys Ala Leu Glu Pro Ser Gly Cys	
365 370 375 380	
act tca cta ccc agc aaa gcc tcc acg agg gca gct cgc ctt gga gag	1428
Thr Ser Leu Pro Ser Lys Ala Ser Thr Arg Ala Ala Arg Leu Gly Glu	
385 390 395	
tac tta cta caa gac ctg cag tca ggc cag tgt ctg cag cta tgg gac	1476
Tyr Leu Leu Gln Asp Leu Gln Ser Gly Gln Cys Leu Gln Leu Trp Asp	
400 405 410	
gat gac ttg gga gcg cta tgg gcc tgc ccc atg gac aaa tac atc cac	1524
Asp Asp Leu Gly Ala Leu Trp Ala Cys Pro Met Asp Lys Tyr Ile His	
415 420 425	
aag cgc tgg gcc ctc gtg tgg ctg gcc tgc cta ctc ttt gcc gct gcg	1572
Lys Arg Trp Ala Leu Val Trp Leu Ala Cys Leu Leu Phe Ala Ala Ala	
430 435 440	
ctt tcc ctc atc ctc ctt ctc aaa aag gat cac gcg aaa ggg tgg ctg	1620
Leu Ser Leu Ile Leu Leu Leu Lys Lys Asp His Ala Lys Gly Trp Leu	
445 450 455 460	
agg ctc ttg aaa cag gac gtc cgc tcg ggg gcg gcc gcc agg ggc cgc	1668
Arg Leu Leu Lys Gln Asp Val Arg Ser Gly Ala Ala Ala Arg Gly Arg	
465 470 475	
gcg gct ctg ctc ctc tac tca gcc gat gac tcg ggt ttc gag cgc ctg	1716
Ala Ala Leu Leu Leu Tyr Ser Ala Asp Asp Ser Gly Phe Glu Arg Leu	
480 485 490	
gtg ggc gcc ctg gcg tcg gcc ctg tgc cag ctg ccg ctg cgc gtg gcc	1764
Val Gly Ala Leu Ala Ser Ala Leu Cys Gln Leu Pro Leu Arg Val Ala	
495 500 505	
gta gac ctg tgg agc cgt cgt gaa ctg agc gcg cag ggg ccc gtg gct	1812
Val Asp Leu Trp Ser Arg Arg Glu Leu Ser Ala Gln Gly Pro Val Ala	
510 515 520	
tgg ttt cac gcg cag cgg cgc cag acc ctg cag gag ggc ggc gtg gtg	1860
Trp Phe His Ala Gln Arg Arg Gln Thr Leu Gln Glu Gly Gly Val Val	
525 530 535 540	
gtc ttg ctc ttc tct ccc ggt gcg gtg gcg ctg tgc agc gag tgg cta	1908
Val Leu Leu Phe Ser Pro Gly Ala Val Ala Leu Cys Ser Glu Trp Leu	
545 550 555	
cag gat ggg gtg tcc ggg ccc ggg gcg cac ggc ccg cac gac gcc ttc	1956
Gln Asp Gly Val Ser Gly Pro Gly Ala His Gly Pro His Asp Ala Phe	
560 565 570	

cgc gcc tcg ctc agc tgc gtg ctg ccc gac ttc ttg cag ggc cgg gcg 2004  
Arg Ala Ser Leu Ser Cys Val Leu Pro Asp Phe Leu Gln Gly Arg Ala  
575 580 585

5      ccc ggc agc tac gtg ggg gcc tgc ttc gac agg ctg ctc cac ccg gac    2052  
Pro Gly Ser Tyr Val Gly Ala Cys Phe Asp Arg Leu Leu His Pro Asp  
               590                          595                          600

10      gcc gta ccc gcc ctt ttc cgc acc gtg ccc gtc ttc aca ctg ccc tcc    2100  
Ala Val Pro Ala Leu Phe Arg Thr Val Pro Val Phe Thr Leu Pro Ser  
605                  610                  615                  620

15      caa ctg cca gac ttc ctg ggg gcc ctg cag cag cct cgc gcc ccg cgt    2148  
         Gln Leu Pro Asp Phe Leu Gly Ala Leu Gln Gln Pro Arg Ala Pro Arg  
                        625                         630                         635

tcc ggg cgg ctc caa gag aga gcg gag caa gtg tcc cgg gcc ctt cag 2196  
Ser Gly Arg Leu Gln Glu Arg Ala Glu Gln Val Ser Arg Ala Leu Gln  
640 645 650

cca gcc ctg gat agc tac ttc cat ccc ccg ggg acn tcc gcg ccg gga 2244  
Pro Ala Leu Asp Ser Tyr Phe His Pro Pro Gly Xaa Ser Ala Pro Gly  
655 660 665

cgc ggg gtg gga cca ggg gcg gga cct ggg gcg ggg gac ggg act 2289  
Arg Gly Val Gly Pro Gly Ala Gly Pro Gly Ala Gly Asp Gly Thr  
670 675 680

taaataaagg cagacgctg 2308

MPVPWFLLSLALGRSQWILSLERLVGPQDATHCSPGLSCRLWSDILCLPGDIVPAPGPVLAPTHLQTELVL  
RCQKETDCDLCLRVAVHLAVHGHWEPEDEEKFGGAADLGVEEPRNASLQAQVVL SFQAYPTARCVLLEVQV  
PAALVQFGQSVGSVVYDCFEAALGSEVRIWSYTPRYEKELNHTQQLPDCRGLLEVWNSIPSCWALPWLNVSA  
DGDNVHLVLNVSEEQHFGLSLYWNQVQGPPKPRWHKNLTGPQIITL NHTDLVPCLCIQVWPLEPDSVRTNIC  
PFREDPRAHQNLWQAARLRLTLQSWLLDAPCSLPAAEALCWRAPGGDPCQPLVPPLSWENVTVDVNSSEKL  
QLQECLWADSLGPLKDDVLLLETRGPQDNRS LCALEPSGCTSLPSKASTRAARLGEYLLQDLQSGQCLQLWD  
DDLGA LWACPMADKYIHKRWALVWLACLFAAALS LILLKKDHAKGWLRL LKQDVRSGAAARGRAALLLYSA  
DDSGFERLVGMDALASALCQLPLRAVDLWSRRELSAQGPVAFHQRRLDTLQEGGVVVL LFSPGAVALCSEWL  
QDGVSGPGAHGPHDAFRASLSCVLPDFLQGRAGPSYVGACFDRLLRPDPAVLFRTVPVFTLPSQLPDFLGA  
LQQPRAPRSGR LQERAEQVSRA LQPALDSYFHPPGTSAPGRGVGPGAGPGAGDGT.

Reverse translation of primate, e.g., human, DCRS7 (SEQ ID NO: 9):

45 atgccngtnc cntgggttyt nytnwsnytn gcnytnggngm gnwsncartg gathytnwsn 60

ytngarmgny tngtnggncc ncargaygcn acncaytgyw snccnggnyt nwsntgymgn 120

50 ytnntgggaww sngayathyt ntgyytnccn ggngayathg tncncgncnc nggncncngtn 180

ytngcncna cncayytnc racngarytn gtnytnmgnt gycaraarga racngaytgy 240

gayytnntgyy tnmngntngc ngtncayytn gcngtncayg gncaytggga rgarccngar 300

55 gaygargara arttyggngg ngcngcngay ytngngngtng argarccnmg naaygcwnsn 360

ytncargcnc argtngtnyt nwsnttycar gcntayccna cngcnmgntg ygtnytnytn 420

gargtncarg tncngcngc nytngtncar ttyggncarw sngtnggnws ngtngtntay 480

gaytgyttyg argcngcnyt nggnwsngar gtnmgnatht ggwsntayac ncarccnmgn 540  
 5 taygaraarg arytnaayca yacncarcar ytncngayt gymngngnyt ngargtntgg 600  
 aaywsnathc cnwsntgytg ggcnytncn tggytnaayg tnwsngcnga yggngayaay 660  
 gtncayytng tnytnaaygt nwsngargar carcayttyg gnytnwsnyt ntaytggaay 720  
 10 cargtncarg gnccnccnaa rccnmgttg cayaaraay tncnggncc ncarathath 780  
 acnytnaayc ayacngayyt ngtnccntgy ytntgyathc argtntggcc nytngarccn 840  
 15 gaywsngtnm gnacnaayat htgyccntty mgngargayc cnmgngcnca ycaraaaytn 900  
 tggcargcng cnmgnytnmg nytnytnacn ytncarwsnt ggytnytnga ygcncntgy 960  
 wsnytnccng cngargcngc nytnygytg mgngcncng gngngaycc ntgycarccn 1020  
 20 ytngtnccnc cnytnwsntg ggaraaygt acngtngayg tnaaywsnws ngaraarytn 1080  
 carytncarg artgyytntg ggngaywsn ytnggncny tnaargayga ygtnytnytn 1140  
 ytngaracnm gnggncnca rgayaaymgn wsnytnygyg cnytngarcc nwsnggntgy 1200  
 25 acnwsnytn cwnsnaargc nwsnacnmgn gcngcnmgny tngngarta yytnytnear 1260  
 gayytnearw snggncartg yytnarytn tgggaygayg ayytnggngc nytnyggcn 1320  
 30 tgyccnatgg ayaartayat hcayaarmgn tgggcnytng tntggytngc ntgyytnytn 1380  
 ttygngcng cnytnwsnyt nathytnytn ytnaaraarg ayaygcnaa rggntggytn 1440  
 mgnytnytna arcargaygt nmgnwsnggn gcngcngcnm gnggmngnc ngcnytnytn 1500  
 35 ytntaywsng cngaygayws nggnttygar mgnytngtng gngcnytnge nwsngcnytn 1560  
 tgyarytn cnytnmgnt ngcngtngay ytntggwsnm gnmngaryt nwsngcncar 1620  
 40 ggncngtng cntggtyca ygcncarmgn mgncaracny tncargargg nggngtngtn 1680  
 gtnytnytn tywsncngg ngcngtngcn ytntgywsng artggytnca rgayggngtn 1740  
 wsnggncng gngcncaygg nccncaygay gcnttymng cwnsnytnws ntgygtnytn 1800  
 45 ccngaytty tncarggnmg ngcncnggn wsntaygtng gngcntgytt ygaymgnytn 1860  
 ytncaycng aygngtnc ngcnytnnty mgnacngtnc cngtnttyac nytnccnwsn 1920  
 50 carytnccng ayttyytngg ngcnytnear carccnmng cncnmgng nngnmngnytn 1980  
 cargarmng cngarcargt nwsnmngcn ytncarccng cnytngayws ntayttyay 2040  
 55 ccncnggna cwnsgncnc nggngnggn gtnggncng gngcnggnc nggngcnggn 2100  
 gayggnacn 2109

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 09063018-052301



ccaaatcgaa agcacgggag ctgatactgg gcctggagtc caggctcact ggagtgggga 60  
agcatggctg gagaggaatt ctagcccttg ctctctccca gggacacggg gctgattgtc 120  
agcagggggcg aggggtctgc ccccccttgg gggggcagga cggggcctca ggcctgggtg 180

cag gac act gca cgc tgc tct cta ggc ctc tcc tgc cac ctc tgg gat 327  
Gln Asp Thr Ala Arg Cys Ser Leu Gly Leu Ser Cys His Leu Trp Asp  
10 15 20

ggt gac gtg ctc tgc ctg cct gga agc ctc cag tct gcc cca ggc cct 375  
Gly Asp Val Leu Cys Leu Pro Gly Ser Leu Gln Ser Ala Pro Gly Pro  
25 30 35

gtg cta gtg cct acc cgc ctg cag acg gag ctg gtg ctg agg tgt cca 423  
Val Leu Val Pro Thr Arg Leu Gln Thr Glu Leu Val Leu Arg Cys Pro  
40 45 50 55

cag aag aca gat tgc gcc ctc tgt gtc cgt gtg gtg gtc cac ttg gcc 471  
Gln Lys Thr Asp Cys Ala Leu Cys Val Arg Val Val Val His Leu Ala  
60 65 70

gtg cat ggg cac tgg gca gag cct gaa gaa gct gga aag tct gat tca 519  
Val His Gly His Trp Ala Glu Pro Glu Glu Ala Gly Lys Ser Asp Ser  
75 80 85

gaa ctc cag gag tct agg aac gcc tct ctc cag gcc cag gtg gtg ctc 567  
Glu Leu Gln Glu Ser Arg Asn Ala Ser Leu Gln Ala Gln Val Val Leu  
90 95 100

tcc	ttc	cag	gcc	tac	ccc	atc	gcc	cgc	tgt	gcc	ctg	ctg	gag	gtc	cag	615
Ser	Phe	Gln	Ala	Tyr	Pro	Ile	Ala	Arg	Cys	Ala	Leu	Leu	Glu	Val	Gln	
	105					110					115					

gtg	ccc	gct	gac	ctg	gtg	cag	cct	ggc	cag	tcc	gtg	ggc	tct	gcg	gta	663
Val	Pro	Ala	Asp	Leu	Val	Gln	Pro	Gly	Gln	Ser	Val	Gly	Ser	Ala	Val	
120					125					130					135	

ttt gac tgt ttc gag gct agt ctt ggg gct gag gta cag atc tgg tcc 711  
Phe Asp Cys Phe Glu Ala Ser Leu Gly Ala Glu Val Gln Ile Trp Ser  
140 145 150

tac acg aag ccc agg tac cag aaa gag ctc aac ctc aca cag cag ctg 759  
 Tyr Thr Lys Pro Arg Tyr Gln Lys Glu Leu Asn Leu Thr Gln Gln Leu  
 155 160 165

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0 5 10 15 20 25 30 35

cct Pro	gac Asp	tgc Cys 170	agg Arg	ggt Gly	ctt Leu	gaa Glu	gtc Val 175	cgg Arg	gac Asp	agc Ser	atc Ile	cag Gln 180	agc Ser	tgc Cys	tgg Trp	807
gtc Val 185	ctg Leu	ccc Pro	tgg Trp	ctc Leu	aat Asn	gtg Val 190	tct Ser	aca Thr	gat Asp	ggt Gly	gac Asp 195	aat Asn	gtc Val	ctt Leu	ctg Leu	855
aca Thr 200	ctg Leu	gat Asp	gtc Val	tct Ser	gag Glu 205	gag Glu	cag Gln	gac Asp	ttt Phe	agc Ser 210	ttc Phe	tta Leu	ctg Leu	tac Tyr	ctg Leu 215	903
cgt Arg	cca Pro	gtc Val	ccg Pro	gat Asp 220	gct Ala	ctc Leu	aaa Lys	tcc Ser	ttg Leu 225	tgg Trp	tac Tyr	aaa Lys	aac Asn	ctg Leu 230	act Thr	951
gga Gly	cct Pro	cag Gln 235	aac Asn	att Ile	act Thr	tta Leu	aac Asn 240	cac His	aca Thr	gac Asp	ctg Leu	gtt Val 245	ccc Pro	tgc Cys	ctc Leu	999
tgc Cys	att Ile 250	cag Gln	gtg Val	tgg Trp	tcg Ser	cta Leu	gag Glu 255	cca Pro	gac Asp	tct Ser	gag Glu	agg Arg 260	gtc Val	gaa Glu	ttc Phe	1047
tgc Cys 265	ccc Pro	ttc Phe	cgg Arg	gaa Glu	gat Asp	ccc Pro 270	ggt Gly	gca Ala	cac His	agg Arg 275	aac Asn	ctc Leu	tgg Trp	cac His	ata Ile	1095
gcc Ala 280	agg Arg	ctg Leu	cgg Arg	gta Val 285	ctg Leu	tcc Ser	cca Pro	ggg Gly	gta Val 290	tgg Trp	cag Gln	cta Leu	gat Asp	gcg Ala 295	cct Pro	1143
tgc Cys	tgt Cys	ctg Leu	ccg Pro	ggc Gly 300	aag Lys	gta Val	aca Thr	ctg Leu 305	tgc Cys	tgg Trp	cag Gln	gca Ala	cca Pro	gac Asp 310	cag Gln	1191
agt Ser	ccc Pro	tgc Cys	cag Gln 315	cca Pro	ctt Leu	gtg Val	cca Pro	cca Pro 320	gtg Val	ccc Pro	cag Gln	aag Lys 325	aac Asn	gcc Ala	act Thr	1239
gtg Val	aat Asn 330	gag Glu	cca Pro	caa Gln	gat Asp	ttc Phe 335	cag Gln	ttg Leu 335	gtg Val	gca Ala	ggc Gly 340	cac His 340	ccc Pro	aac Asn	ctc Leu	1287
tgt Cys 345	gtc Val	cag Gln	gtg Val	agc Ser	acc Thr	tgg Trp 350	gag Glu	aag Lys	gtt Val	cag Gln	ctg Leu 355	caa Gln	gcg Ala	tgc Cys	ttg Leu	1335
tgg Trp 360	gct Ala	gac Asp	tcc Ser	ttg Leu	ggg Gly 365	ccc Pro	ttc Phe	aag Lys	gat Asp	gat Asp 370	atg Met	ctg Leu	tta Leu	gtg Val	gag Glu 375	1383
atg Met	aaa Lys	acc Thr	ggc Gly	ctc Leu 380	aac Asn	aac Asn	aca Thr	tca Ser	gtc Val 385	tgt Cys	gcc Ala	ttg Leu	gaa Glu	ccc Pro 390	agt Ser	1431
ggc Gly	tgt Cys	aca Thr	cca Pro 395	ctg Leu	ccc Pro	agc Ser	atg Met	gcc Ala 400	tcc Ser	acg Thr	aga Arg	gct Ala 405	gct Ala	cgc Arg	ctg Leu	1479



tcc act tcc gcg ggg cga ccc gcg gac cgg gtg gaa cga gtg acc cag 2199  
 Ser Thr Ser Ala Gly Arg Pro Ala Asp Arg Val Glu Arg Val Thr Gln  
 635 640 645

5 gcg ctg cgg tcc gcc ctg gac agc tgt act tct agc tcg gaa gcc cca 2247  
 Ala Leu Arg Ser Ala Leu Asp Ser Cys Thr Ser Ser Glu Ala Pro  
 650 655 660

10 ggc tgc tgc gag gaa tgg gac ctg gga ccc tgc act aca cta gaa 2292  
 Gly Cys Cys Glu Glu Trp Asp Leu Gly Pro Cys Thr Thr Leu Glu  
 665 670 675

taaaagccga tacagtattc ct 2314

15 MPVSWFLLSLALGRNPVVVSLERLMEPQDTARCSLGLSCHLWDGDVLCPLPGSLQSAPGPVLVPTRLQTELVL  
 RCPQKTDICALCVRVVVHLAVHGHWAEPPEEAGKSDSELQESRNASLQAQVVL SFQAYPIARCALLEVQVPADL  
 VQPGQSVGSAVFDCFEASLGAEVQIWSYTKPRYQKELNLTQQLPDCRGLEVRDSIQSCWVLPWLVNSTDGDN  
 VLLTLDVSEEQDFSFLLYLRPVPDALKSLWYKNLTGPNITLNHTDLVPCLCIQVWSLEPDSEVEFCPFRE  
 DPGAHRNLWHIARLRVLSPGVWQLDAPCCLPGKVTLCWQAPDQSPCQPLVPPVPQKNATVNEPQDFQLVAGH  
 PNLVCQVSTWEKVQLQACLWADSLGPFKDDMLLVEMKTGLNNTSVCALEPSGCTPLPSMASTRAARLGEELL  
 QDFRSHQCMQLWNDDNMGSLWACPMCKYIHRRWLVWLACLLAAALFFFLLLKKDRKAARGSR TALLLHS  
 ADGAGYERLVGALASALSQMPLRVAVDLWSRRELSAHGALAWFHHQRRRILQEGGVVILLFSPAAVAQCQW  
 LQLQTVEPGPHDALAAWLSCVLPDFLQGRATGRYVGVYFDGLLHPDSVPSPPFRVAPLFLSLPSQLPAFLDALQ  
 GGCSTSAGRPADRVERVTQALRSALDSCSTSSSEAPGCCCEWDLGPCTTLE.

Reverse translation of rodent, e.g., mouse, DCRS7 (SEQ ID NO: 12):

atgccngtnw sntgggttyt nytnwsnytn gcnytnngnm gnaayccngt ngtnngtnwsn 60  
 ytnngarmgny tnatggarcc ncargayacn gcnmgntgyw snytnnggnyt nwsntgy cay 120  
 ytntgggag gngaygtnyt ntgyytnccn ggnwsnytn arwsngcncc nggnccngtn 180  
 ytngtncna cnmgnytnca racngarytn gtnytnmgnt gyccncaraa racngaytgy 240  
 gcnytnngy tnmngntngt ngtncaaytn gcngtncaay gncaytgggc ngarccngar 300  
 gargcnggna arwsngayws ngarytnca garwsnmgna aygcwnsnyt ncargcncar 360  
 gtngtnytnw snttycargc ntayccnath gcnmgntgyg cnytnytn ga rgtncargtn 420  
 ccngcngayy tngtnca rcc nggncarwsn gtnggnwsng cngtnnttyga ytgyttygar 480  
 gcwnsnytn gngcngargt ncarathtg wsntayacna arccnmngnta ycaraargar 540  
 ytnaayytna cncarcaryt nccngaytgy mgnggnytn argtnmgnga ywsnathcar 600  
 wsntgytggg tnytnccntg gytnaaytn wsnacngay gngayaaygt nytnytnacn 660  
 ytngaygtnw sngargarca rgayttywsn tyytnytn ayytnmgnc ngtnccngay 720  
 gcnytnaarw snytnngta yaaraaytn acnggncnc araayathac nytnaaycay 780  
 acngayytn tncntgyt ntgyathcar gtntggwsny tngarccnga ywsngarmgn 840  
 gtngarttyt gyccnttymg ngargayccn ggngcncaym gnaayytn gcaayathcn 900  
 mgnytnmgng tnytnwsncc ngngntngt carytngay cncntgyt yytnccnggn 960

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aargtnacny tntgytgga rgcnccngay carwsnccnt gycarccnyt ngtnccnccn 1020  
 gtnccncara araaygcna ngtnaaygar ccncargayt tycarytngt ngcnngncay 1080  
 ccnaayytnt gygtncargt nwsnacntgg garaargtnc arytnccargc ntgyytntgg 1140  
 gcngaywsny tnggnccntt yaargaygay atgytntng tngaratgaa racnggnytn 1200  
 aayaayacnw sngtntgygc nytngarccn wsgngntgya cncnytncc nwsnatggcn 1260  
 wsnacnmng cngcnmngnt ngngargar ytnytnccarg ayttymgnws ncaycartgy 1320  
 atgcarytnt ggaaygayga yaayatgggn wsnytnctgg cntgyccnat ggayaartay 1380  
 athcaymngm gntgggntyt ngtnctgytn gcntgyytny tnytnccngc ngcnytncty 1440  
 ttyttyytny tnytnaaraa rgaymngmgn aargcngcnm gnggnwsnmg nacngcnnytn 1500  
 ytnytnccayw sngcngaygg ngcnngntay garmgnytn tngngcncty ngcnwsnccn 1560  
 ytnwsncara tgccnytnmg ngtnccngtn gayytnctggw snmngmngna rytwnsnccn 1620  
 cayggngcnny tngcntggtt ycaycaycar mngmngmngna thytnccarg rggngngntn 1680  
 gtnathytny tnttywsncc ngcnccngtn gncartgyc arcartggyt ncarytnccar 1740  
 acngtnccar cnggnccnca ygaygcnytn gngcntggy twnsntgygt nytnccngay 1800  
 ttyytnccarg gnmngcnac nggnmngntay gtnngngntt aytygaygg nytnytnccay 1860  
 ccngaywsng tncnwsncc nttymngntn gncnytnct tywsnytncc nwsncarytn 1920  
 ccngcnttyy tngaygcnytn ncarggnggn tgywsnacnw sngcngngmng nccngcngay 1980  
 mngntngarm gngtnacna rcnytnmngn wsgcnnytn aywsntgyac nwsnwsnwn 2040  
 gargcncng gntgytgyga rgartgggay ytnngnccnt gyacnacnytn ngar 2094

Table 3: Nucleotide and polypeptide sequences of DNAX Cytokine Receptor Subunit like  
 embodiments (DCRS8). Primate, e.g., human, embodiment (see SEQ ID NO: 13 and 14).  
 Predicted signal sequence indicated, but may vary by a few positions and depending upon cell  
 type.

cccacgcntc cgggccagca gcgggcggcc ggggcgcaga gaacggcctg gctgggcgag 60  
 cgcacggcc atg gcc ccg tgg ctg cag ctc tgc tcc gtc ttc ttt acg gtc 111  
 Met Ala Pro Trp Leu Gln Leu Cys Ser Val Phe Phe Thr Val  
 -15 -10 -5  
 aac gcc tgc ctc aac ggc tcg cag ctg gct gtn gcc gct ggc ggg tcc 159  
 Asn Ala Cys Leu Asn Gly Ser Gln Leu Ala Xaa Ala Ala Gly Gly Ser  
 -1 1 5 10  
 ggc cgc gcg cng ggc gcc gac acc tgt agc tgg ang gga gtg ggg cca 207  
 Gly Arg Ala Xaa Gly Ala Asp Thr Cys Ser Trp Xaa Gly Val Gly Pro  
 15 20 25 30

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5	gcc agc aga aac agt ggg ctg tac aac atc acc ttc aaa tat gac aat Ala Ser Arg Asn Ser Gly Leu Tyr Asn Ile Thr Phe Lys Tyr Asp Asn	255
	35 40 45	
10	tgt acc acc tac ttg aat cca gtg ggg aag cat gtg att gct gac gcc Cys Thr Thr Tyr Leu Asn Pro Val Gly Lys His Val Ile Ala Asp Ala	303
	50 55 60	
15	cag aat atc acc atc agc cag tat gct tgc cat gac caa gtg gca gtc Gln Asn Ile Thr Ile Ser Gln Tyr Ala Cys His Asp Gln Val Ala Val	351
	65 70 75	
20	acc att ctt tgg tcc cca ggg gcc ctc ggc atc gaa ttc ctg aaa gga Thr Ile Leu Trp Ser Pro Gly Ala Leu Gly Ile Glu Phe Leu Lys Gly	399
	80 85 90	
25	ttt cgg gta ata ctg gag gag ctg aag tcg gag gga aga cag ngc caa Phe Arg Val Ile Leu Glu Glu Leu Lys Ser Glu Gly Arg Gln Xaa Gln	447
	95 100 105 110	
30	caa ctg att cta aag gat ccg aag cag ntc aac agt agc ttc aaa aga Gln Leu Ile Leu Lys Asp Pro Lys Gln Xaa Asn Ser Ser Phe Lys Arg	495
	115 120 125	
35	act gga atg gaa tct caa cct ttn ctg aat atg aaa ttt gaa acg gat Thr Gly Met Glu Ser Gln Pro Xaa Leu Asn Met Lys Phe Glu Thr Asp	543
	130 135 140	
40	tat ttc gta agg ttg tcc ttt tcc ttc att aaa aac gaa agc aat tac Tyr Phe Val Arg Leu Ser Phe Ser Phe Ile Lys Asn Glu Ser Asn Tyr	591
	145 150 155	
45	cac cct ttc ttc ttt aga acc cga gcc tgt gac ctg ttg tta cag ccg His Pro Phe Phe Phe Arg Thr Arg Ala Cys Asp Leu Leu Leu Gln Pro	639
	160 165 170	
50	gac aat cta gct tgt aaa ccc ttc tgg aag cct cgg aac ctg aac atc Asp Asn Leu Ala Cys Lys Pro Phe Trp Lys Pro Arg Asn Leu Asn Ile	687
	175 180 185 190	
55	agc cag cat ggc tcg gac atg cag gtg tcc ttc gac cac gca ccg cac Ser Gln His Gly Ser Asp Met Gln Val Ser Phe Asp His Ala Pro His	735
	195 200 205	
60	aac ttc ggc ttc cgt ttc ttc tat ctt cac tac aag ctc aag cac gaa Asn Phe Gly Phe Arg Phe Phe Tyr Leu His Tyr Lys Leu Lys His Glu	783
	210 215 220	
65	gga cct ttc aag cga aag acc tgt aag cag gag caa act aca gag atg Gly Pro Phe Lys Arg Lys Thr Cys Lys Gln Glu Gln Thr Thr Glu Met	831
	225 230 235	
70	acc agc tgc ctc ctt caa aat gtt tct cca ggg gat tat ata att gag Thr Ser Cys Leu Leu Gln Asn Val Ser Pro Gly Asp Tyr Ile Ile Glu	879
	240 245 250	

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	ctg	gtg	gat	gac	act	aac	aca	aca	aga	aaa	gtg	atg	cat	tat	gcc	tta	927
	Leu	Val	Asp	Asp	Thr	Asn	Thr	Thr	Arg	Lys	Val	Met	His	Tyr	Ala	Leu	
	255					260					265					270	
5	aag	cca	gtg	cac	tcc	ccg	tgg	gcc	ggg	ccc	atc	aga	gcc	gtg	gcc	atc	975
	Lys	Pro	Val	His	Ser	Pro	Trp	Ala	Gly	Pro	Ile	Arg	Ala	Val	Ala	Ile	
					275					280					285		
10	aca	gtg	cca	ctg	gta	gtc	ata	tcg	gca	ttc	gcg	acg	ctc	ttc	act	gtg	1023
	Thr	Val	Pro	Leu	Val	Val	Ile	Ser	Ala	Phe	Ala	Thr	Leu	Phe	Thr	Val	
				290					295					300			
15	atg	tgc	cgc	aag	aag	caa	caa	gaa	aat	ata	tat	tca	cat	tta	gat	gaa	1071
	Met	Cys	Arg	Lys	Lys	Gln	Gln	Glu	Asn	Ile	Tyr	Ser	His	Leu	Asp	Glu	
			305					310					315				
	gag	agc	tct	gag	tct	tcc	aca	tac	act	gca	gca	ctc	cca	aga	gag	agg	1119
	Glu	Ser	Ser	Glu	Ser	Ser	Thr	Tyr	Thr	Ala	Ala	Leu	Pro	Arg	Glu	Arg	
		320					325					330					
20	ctc	cgg	ccg	cgg	ccg	aag	gtc	ttt	ctc	tgc	tat	tcc	agt	aaa	gat	ggc	1167
	Leu	Arg	Pro	Arg	Pro	Lys	Val	Phe	Leu	Cys	Tyr	Ser	Ser	Lys	Asp	Gly	
	335					340					345					350	
25	cag	aat	cac	atg	aat	gtc	gtc	cag	tgt	ttc	gcc	tac	ttc	ctc	cag	gac	1215
	Gln	Asn	His	Met	Asn	Val	Val	Gln	Cys	Phe	Ala	Tyr	Phe	Leu	Gln	Asp	
					355					360					365		
30	ttc	tgt	ggc	tgt	gag	gtg	gct	ctg	gac	ctg	tgg	gaa	gac	ttc	agc	ctc	1263
	Phe	Cys	Gly	Cys	Glu	Val	Ala	Leu	Asp	Leu	Trp	Glu	Asp	Phe	Ser	Leu	
				370					375					380			
35	tgt	aga	gaa	ggg	cag	aga	gaa	tgg	gtc	atc	cag	aag	atc	cac	gag	tcc	1311
	Cys	Arg	Glu	Gly	Gln	Arg	Glu	Trp	Val	Ile	Gln	Lys	Ile	His	Glu	Ser	
			385					390					395				
	cag	ttc	atc	att	gtg	gtt	tgt	tcc	aaa	ggg	atg	aag	tac	ttt	gtg	gac	1359
	Gln	Phe	Ile	Ile	Val	Val	Cys	Ser	Lys	Gly	Met	Lys	Tyr	Phe	Val	Asp	
		400					405					410					
40	aag	aag	aac	tac	aaa	cac	aaa	gga	ggg	ggc	cga	ggc	tcg	ggg	aaa	gga	1407
	Lys	Lys	Asn	Tyr	Lys	His	Lys	Gly	Gly	Gly	Arg	Gly	Ser	Gly	Lys	Gly	
	415					420					425					430	
45	gag	ctc	ttc	ctg	gtg	gcg	gtg	tca	gcc	att	gcc	gaa	aag	ctc	cgc	cag	1455
	Glu	Leu	Phe	Leu	Val	Ala	Val	Ser	Ala	Ile	Ala	Glu	Lys	Leu	Arg	Gln	
					435				440						445		
50	gcc	aag	cag	agt	tcg	tcc	gcg	gcg	ctc	agc	aag	ttt	atc	gcc	gtc	tac	1503
	Ala	Lys	Gln	Ser	Ser	Ser	Ala	Ala	Leu	Ser	Lys	Phe	Ile	Ala	Val	Tyr	
				450					455					460			
	ttt	gat	tat	tcc	tgc	gag	gga	gac	gtc	ccc	ggg	atc	cta	gac	ctg	agt	1551
	Phe	Asp	Tyr	Ser	Cys	Glu	Gly	Asp	Val	Pro	Gly	Ile	Leu	Asp	Leu	Ser	
			465					470					475				
55	acc	aag	tac	aga	ctc	atg	gac	aat	ctt	cct	cag	ctc	tgt	tcc	cac	ctg	1599
	Thr	Lys	Tyr	Arg	Leu	Met	Asp	Asn	Leu	Pro	Gln	Leu	Cys	Ser	His	Leu	
		480					485					490					

cac tcc cga gac cac ggc ctc cag gag ccg ggg cag cac acg cga cag	1647
His Ser Arg Asp His Gly Leu Gln Glu Pro Gly Gln His Thr Arg Gln	
495 500 505 510	
ggc agc aga agg aac tac ttc cgg agc aag tca ggc cgg tcc cta tac	1695
Gly Ser Arg Arg Asn Tyr Phe Arg Ser Lys Ser Gly Arg Ser Leu Tyr	
515 520 525	
gtc gcc att tgc aac atg cac cag ttt att gac gag gag ccc gac tgg	1743
Val Ala Ile Cys Asn Met His Gln Phe Ile Asp Glu Glu Pro Asp Trp	
530 535 540	
ttc gaa aag cag ttc gtt ccc ttc cat cct cct cca ctg cgc tac cgg	1791
Phe Glu Lys Gln Phe Val Pro Phe His Pro Pro Pro Leu Arg Tyr Arg	
545 550 555	
gag cca gtc ttg gag aaa ttt gat tgc ggc ttg gtt tta aat gat gtc	1839
Glu Pro Val Leu Glu Lys Phe Asp Ser Gly Leu Val Leu Asn Asp Val	
560 565 570	
atg tgc aaa cca ggg cct gag agt gac ttc tgc cta aag gta gag gcg	1887
Met Cys Lys Pro Gly Pro Glu Ser Asp Phe Cys Leu Lys Val Glu Ala	
575 580 585 590	
gct gtt ctt ggg gca acc gga cca gcc gac tcc cag cac gag agt cag	1935
Ala Val Leu Gly Ala Thr Gly Pro Ala Asp Ser Gln His Glu Ser Gln	
595 600 605	
cat ggg ggc ctg gac caa gac ggg gag gcc cgg cct gcc ctt gac ggt	1983
His Gly Gly Leu Asp Gln Asp Gly Glu Ala Arg Pro Ala Leu Asp Gly	
610 615 620	
agc gcc gcc ctg caa ccc ctg ctg cac acg gtg aaa gcc ggc agc ccc	2031
Ser Ala Ala Leu Gln Pro Leu Leu His Thr Val Lys Ala Gly Ser Pro	
625 630 635	
tcg gac atg ccg cgg gac tca ggc atc tat gac tcg tct gtg ccc tca	2079
Ser Asp Met Pro Arg Asp Ser Gly Ile Tyr Asp Ser Ser Val Pro Ser	
640 645 650	
tcc gag ctg tct ctg cca ctg atg gaa gga ctc tcg acg gac cag aca	2127
Ser Glu Leu Ser Leu Pro Leu Met Glu Gly Leu Ser Thr Asp Gln Thr	
655 660 665 670	
gaa acg tct tcc ctg acg gag agc gtg tcc tcc tct tca ggc ctg ggt	2175
Glu Thr Ser Ser Leu Thr Glu Ser Val Ser Ser Ser Ser Gly Leu Gly	
675 680 685	
gag gag gaa cct cct gcc ctt cct tcc aag ctc ctc tct tct ggg tca	2223
Glu Glu Glu Pro Pro Ala Leu Pro Ser Lys Leu Leu Ser Ser Gly Ser	
690 695 700	
tgc aaa gca gat ctt ggt tgc cgc agc tac act gat gaa ctc cac gcg	2271
Cys Lys Ala Asp Leu Gly Cys Arg Ser Tyr Thr Asp Glu Leu His Ala	
705 710 715	
gtc gcc cct ttg taacaaaacg aaagagtcta agcattgccca ctttagctgc	2323
Val Ala Pro Leu	
720	



5 tgctccctc tgattcccca gctcatctcc ctggttgcag ggcacttg gagctgaggt 2383  
 ctcatacaag gatatttgga gtgaaatgct ggccagtact tgttctccct tgccccaacc 2443  
 ctttaccgga tatcttgaca aactctccaa ttttctaaaa tgatatggag ctctgaaagg 2503  
 catgtccata aggtctgaca acagcttgcc aaatttggtt agtccttgga tcagagcctg 2563  
 10 ttgtgggagg tagggaggaa atatgtaaag aaaaacagga agatacctgc actaatcatt 2623  
 cagacttcat tgagctctgc aaactttgcc tgtttgctat tggctacctt gatttgaaat 2683  
 15 gctttgtgaa aaaaggcact ttttaacatca tagccacaga aatcaagtgc cagtctatct 2743  
 ggaatccatg ttgtattgca gataatgttc tcatttattt ttg 2786

MAPWLQLCSVFFTVNACLNGSQLAVAAGGSGRAXGADTCSWXGVGPASRNSGLYNITFKYDNCTTYLNPVGK  
 HVIADAQNITISQYACHDQVAVTILWSPGALGIEFLKGRVILEELKSEGRQXQQLILKDPKQXNSSFKRTG  
 MESQPXLNMKFETDYFVRLSFSFIKNESNYHPFFRTRACDLLQPDNLACKPFWKPRNLNISQHGSDMQVS  
 FDHAPHNFGFRFFYLHYLKHGEGPFKRKTCKQEQTTEMTSCLLQNVSPGDYIIELVDDTNTTRKVMHYALKP  
 VHSPWAGPIRAVAITVPLVVISAFATLFTVMCRKKQENIYSHLDEESSESSTYTAALPRERLRPRPKVFLC  
 YSSKDGQNHMNVVQCFAYFLQDFCGCEVALDLWEDFSLCREGQREWVIQKIHESQFIIIVCSKGMKYFVDKK  
 NYKHKGGRSGSGKELFLVAVSAIAEKLKQAKQSSSAALSKFIAVYFDYSCEGDVPGILDSTKYRLMDNLP  
 QLCSHLHSRDHGLQEPGQHTRQGSRRNYFRSKSGRSLYVAICNMHQFIDEEPWFQKQFVFPFPPPLRYREP  
 VLEKFDGLVNDVMCKPGPESDFCLKVEAAVLGATGPADSQHSQHGGLDQDGEARPALDGSAAALQPLLHT  
 VKAGSPSDMPRDSGIYDSSVPSSLSLPLMEGLSTDQTETSSLTESVSSSSGLGEEPPALPSKLLSSGSCK  
 ADLGCRSYDELHAVAPL.

Reverse translation of primate, e.g., human, DCRS8 (SEQ ID NO: 15):

atggencnt ggytnaryt ntgywsngtn ttytthyacng tnaaygcntg yytnaayggn 60  
 wsnarytng cngtngcngc ngngngnwsn ggnmgngcnn nngngngnga yacntgywsn 120  
 tggnnngngng tnggncngc nwsnmgnaay wsngngnyntnt ayaayathac nttyaartay 180  
 40 gayaaytgya cnactayyt naayccngtn ggnaarcayg tnathgcnga ygcncaraay 240  
 athacnathw sncartaygc ntgycaygay cargtngcng tnacnathyt ntggwsnccn 300  
 ggngcnytn gnatthgartt yytnaarggn ttmngngtna thytnngarga rytnaarwsn 360  
 45 gargngmgnc arnnncarca rytnathyt aargayccna arcarnnnnaa ywsnwsntty 420  
 aarmgnacng gnatggarws ncarccnnnn ytnaayatga arttygarac ngaytaytty 480  
 gtnmgnytnw snttywsntt yathaaraay garwsnaayt aycayccntt ytttytymgn 540  
 50 acnmngngent gygayytnt nytnarccn gayaayytng cntgyaarcc nttytggaar 600  
 ccnmgnaay tnaayathws ncarcayggn wsngayatgc argtnwsntt ygaycaygcn 660  
 55 ccncayaayt tyggnttymg nttytthyay ytncaytaya arytnaarca ygargngcnn 720  
 ttyaarmgna aracntgyaa rcargarcar acnacngara tgacnwsntg yytnytnar 780  
 aaygtnwsnc cngngayta yathathgar ytngtngayg ayacnaayac nacnmgnaar 840

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 20  
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 30  
 35

5 gtnatgcayt aygcnytnaa rccngtncay wsnccntggg cnggnccnat hmgngcngtn 900  
 gcnathacng tncnytngt ngtnathwsn genttygcna cnytnntyac ngtnatgtgy 960  
 mgnaraarc arcargaraa yathtaywsn cayytngayg argarwsnws ngarwsnwsn 1020  
 10 acntayacng cngcnytncc nmngngarmgn ytnmgncnm gncnaargt nttyytnngy 1080  
 taywsnwsna argayggna raaycaytg aaygtngtnc artgyttygc ntayttyytn 1140  
 cargayttyt gyggntgyga rgtngcnytn gayytnnggg argayttyws nytntgymgn 1200  
 15 gargncarm gngartgggt nathcaraar athcaygarw sncarttyat hathgtngtn 1260  
 tgywsnaarg gnatgaarta ytygtngay aaraaraayt ayaarcayaa rggngngngn 1320  
 mgnggnwsng gnaarggnga rytnttyytn gtngcngtnw sngcnathgc ngaraarytn 1380  
 20 mgncargcna arcarwsnws nwsngcngcn ytnwsnaart tyathgcngt ntayttygay 1440  
 taywsntgyg arggngaygt nccnggnath ytngayytnw snacnaarta ymgnytnatg 1500  
 25 gayaaytnc encarytnng ywsncayytn caywsnmng ayayggnyt ncargarccn 1560  
 ggncarcaya cnmngcargg nwsnmngmgn aaytaytym gnwsnaarws ngngmgnwsn 1620  
 ytnaygtng cnathtgyaa yatgcaycar ttyathgayg argarccnga ytggttygar 1680  
 30 aarcarttyg tncnttyca yccncncn ytnmgntaym ngarccngt nytnagaraar 1740  
 ttygaywsng gnytnngnyt naaygaygn atgtgyaarc cnggnccnga rwsngaytty 1800  
 35 tgyytnaarg tngargcngc ngtnytnngn gcnacnggnc cngcngayws ncarcaygar 1860  
 wsnarcayg gnggnytna ycargayggg gargcnmgnc cngcnytna yggngwsngcn 1920  
 gcnynarc cnytnytnca yacngtnaar gnggnwsnc cnwsngayat gccnmngay 1980  
 40 wsnggnatht aygaywsnws ngtnccnwsn wsngarytnw snytnccnyt natggarggn 2040  
 ytnwsnacng aycaracnga racnwsnwsn ytnacngarw sngtnwsnws nwsnwsnggn 2100  
 ytnngngarg argarccnc ngcnytnccn wsnaarytny tnwsnwsngg nwsntgyaar 2160  
 45 gcngayytn gntgymgnws ntayacngay garytnayg cngtngcnc nytn 2214

50 Table 4: Nucleotide and polypeptide sequences of DNAX Cytokine Receptor Subunit like  
 embodiments (DCRS9). Primate, e.g., human, embodiment (see SEQ ID NO: 16 and 17).  
 Predicted signal sequence indicated, but may vary by a few positions and depending upon cell  
 type.

55 atg ggg agc tcc aga ctg gca gcc ctg ctc ctg cct ctc ctc ctc ata 48  
 Met Gly Ser Ser Arg Leu Ala Ala Leu Leu Leu Pro Leu Leu Leu Ile  
 -20 -15 -10

	gtc atc gac ctc tct gac tct gct ggg att ggc ttt cgc cac ctg ccc	96
	Val Ile Asp Leu Ser Asp Ser Ala Gly Ile Gly Phe Arg His Leu Pro	
	-5 -1 1 5	
5	cac tgg aac acc cgc tgt cct ctg gcc tcc cac acg gaa gtt ctg cct	144
	His Trp Asn Thr Arg Cys Pro Leu Ala Ser His Thr Glu Val Leu Pro	
	10 15 20 25	
10	ata tcc ctt gcc gca cct ggt ggg ccc tct tct cca caa agc ctt ggt	192
	Ile Ser Leu Ala Ala Pro Gly Gly Pro Ser Ser Pro Gln Ser Leu Gly	
	30 35 40	
15	gtg tgc gag tct ggc act gtt ccc gct gtt tgt gcc agc atc tgc tgt	240
	Val Cys Glu Ser Gly Thr Val Pro Ala Val Cys Ala Ser Ile Cys Cys	
	45 50 55	
20	cag gtg gct cag gtc ttc aac ggg gcc tct tcc acc tcc tgg tgc aga	288
	Gln Val Ala Gln Val Phe Asn Gly Ala Ser Ser Thr Ser Trp Cys Arg	
	60 65 70	
25	aat cca aaa agt ctt cca cat tca agt tct ata gga gac aca aga tgc	336
	Asn Pro Lys Ser Leu Pro His Ser Ser Ser Ile Gly Asp Thr Arg Cys	
	75 80 85	
30	cag cac ctg ctc aga gga agc tgc tgc ctc gtc gtc acc tgt ctg aga	384
	Gln His Leu Leu Arg Gly Ser Cys Cys Leu Val Val Thr Cys Leu Arg	
	90 95 100 105	
35	aga gcc atc aca ttt cca tcc cct ccc cag aca tct ccc aca agg gac	432
	Arg Ala Ile Thr Phe Pro Ser Pro Pro Gln Thr Ser Pro Thr Arg Asp	
	110 115 120	
40	ttc gct cta aaa gga ccc aac ctt cgg atc cag aga cat ggg aaa gtc	480
	Phe Ala Leu Lys Gly Pro Asn Leu Arg Ile Gln Arg His Gly Lys Val	
	125 130 135	
45	ttc cca gat tgg act cac aaa ggc atg gag gtg ggc act ggg tac aac	528
	Phe Pro Asp Trp Thr His Lys Gly Met Glu Val Gly Thr Gly Tyr Asn	
	140 145 150	
50	agg aga tgg gtt cag ctg agt ggt gga ccc gag ttc tcc ttt gat ttg	576
	Arg Arg Trp Val Gln Leu Ser Gly Gly Pro Glu Phe Ser Phe Asp Leu	
	155 160 165	
55	ctg cct gag gcc cgg gct att cgg gtg acc ata tct tca ggc cct gag	624
	Leu Pro Glu Ala Arg Ala Ile Arg Val Thr Ile Ser Ser Gly Pro Glu	
	170 175 180 185	
60	gtc agc gtg cgt ctt tgt cac cag tgg gca ctg gag tgt gaa gag ctg	672
	Val Ser Val Arg Leu Cys His Gln Trp Ala Leu Glu Cys Glu Glu Leu	
	190 195 200	
65	agc agt ccc tat gat gtc cag aaa att gtg tct ggg ggc cac act gta	720
	Ser Ser Pro Tyr Asp Val Gln Lys Ile Val Ser Gly Gly His Thr Val	
	205 210 215	
70	gag ctg cct tat gaa ttc ctt ctg ccc tgt ctg tgc ata gag gca tcc	768
	Glu Leu Pro Tyr Glu Phe Leu Leu Pro Cys Leu Cys Ile Glu Ala Ser	
	220 225 230	

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5	tac ctg caa gag gac act gtg agg cgc aaa aaa tgt ccc ttc cag agc Tyr Leu Gln Glu Asp Thr Val Arg Arg Lys Lys Cys Pro Phe Gln Ser 235 240 245	816
10	tgg cca gaa gcc tat ggc tgc gac ttc tgg aag tca gtg cac ttc act Trp Pro Glu Ala Tyr Gly Ser Asp Phe Trp Lys Ser Val His Phe Thr 250 255 260 265	864
15	gac tac agc cag cac act cag atg gtc atg gcc ctg aca ctc cgc tgc Asp Tyr Ser Gln His Thr Gln Met Val Met Ala Leu Thr Leu Arg Cys 270 275 280	912
20	cca ctg aag ctg gaa gct gcc ctc tgc cag agg cac gac tgg cat acc Pro Leu Lys Leu Glu Ala Ala Leu Cys Gln Arg His Asp Trp His Thr 285 290 295	960
25	ctt tgc aaa gac ctc ccg aat gcc acg gct cga gag tca gat ggg tgg Leu Cys Lys Asp Leu Pro Asn Ala Thr Ala Arg Glu Ser Asp Gly Trp 300 305 310	1008
30	tat gtt ttg gag aag gtg gac ctg cac ccc cag ctc tgc ttc aag gta Tyr Val Leu Glu Lys Val Asp Leu His Pro Gln Leu Cys Phe Lys Val 315 320 325	1056
35	caa cca tgg ttc tct ttt gga aac agc agc cat gtt gaa tgc ccc cac Gln Pro Trp Phe Ser Phe Gly Asn Ser Ser His Val Glu Cys Pro His 330 335 340 345	1104
40	cag act ggg tct ctc aca tcc tgg aat gta agc atg gat acc caa gcc Gln Thr Gly Ser Leu Thr Ser Trp Asn Val Ser Met Asp Thr Gln Ala 350 355 360	1152
45	cag cag ctg att ctt cac ttc tcc tca aga atg cat gcc acc ttc agt Gln Gln Leu Ile Leu His Phe Ser Ser Arg Met His Ala Thr Phe Ser 365 370 375	1200
50	gct gcc tgg agc ctc cca ggc ttg ggg cag gac act ttg gtg ccc ccc Ala Ala Trp Ser Leu Pro Gly Leu Gly Gln Asp Thr Leu Val Pro Pro 380 385 390	1248
55	gtg tac act gtc agc cag gtg tgg cgg tca gat gtc cag ttt gcc tgg Val Tyr Thr Val Ser Gln Val Trp Arg Ser Asp Val Gln Phe Ala Trp 395 400 405	1296
60	aag cac ctc ttg tgt cca gat gtc tct tac aga cac ctg ggg ctc ttg Lys His Leu Leu Cys Pro Asp Val Ser Tyr Arg His Leu Gly Leu Leu 410 415 420 425	1344
65	atc ctg gca ctg ctg gcc ctc ctc acc cta ctg ggt gtt gtt ctg gcc Ile Leu Ala Leu Leu Ala Leu Leu Thr Leu Leu Gly Val Val Leu Ala 430 435 440	1392
70	ctc acc tgc cgg cgc cca cag tca ggc ccg ggc cca gcg cgg cca gtg Leu Thr Cys Arg Arg Pro Gln Ser Gly Pro Gly Pro Ala Arg Pro Val 445 450 455	1440

	ctc ctc ctg cac gcg gcg gac tcg gag gcg cag cgg cgc ctg gtg gga	1488
	Leu Leu Leu His Ala Ala Asp Ser Glu Ala Gln Arg Arg Leu Val Gly	
	460 465 470	
5	gcg ctg gct gaa ctg cta cgg gca gcg ctg ggc ggc ggc cgc gac gtg	1536
	Ala Leu Ala Glu Leu Leu Arg Ala Ala Leu Gly Gly Gly Arg Asp Val	
	475 480 485	
10	atc gtg gac ctg tgg gag ggg agg cac gtg gcg cgc gtg ggc ccg ctg	1584
	Ile Val Asp Leu Trp Glu Gly Arg His Val Ala Arg Val Gly Pro Leu	
	490 495 500 505	
15	ccg tgg ctc tgg gcg gcg cgg acg cgc gta gcg cgg gag cag ggc act	1632
	Pro Trp Leu Trp Ala Ala Arg Thr Arg Val Ala Arg Glu Gln Gly Thr	
	510 515 520	
	gtg ctg ctg ctg tgg agc ggc gcc gac ctt cgc ccg gtc agc ggc ccc	1680
	Val Leu Leu Leu Trp Ser Gly Ala Asp Leu Arg Pro Val Ser Gly Pro	
	525 530 535	
	gac ccc cgc gcc gcg ccc ctg ctc gcc ctg ctc cac gct gcc ccg cgc	1728
	Asp Pro Arg Ala Ala Pro Leu Leu Ala Leu Leu His Ala Ala Pro Arg	
	540 545 550	
25	ccg ctg ctg ctg ctc gct tac ttc agt cgc ctc tgc gcc aag ggc gac	1776
	Pro Leu Leu Leu Leu Ala Tyr Phe Ser Arg Leu Cys Ala Lys Gly Asp	
	555 560 565	
30	atc ccc ccg ccg ctg cgc gcc ctg ccg cgc tac cgc ctg ctg cgc gac	1824
	Ile Pro Pro Pro Leu Arg Ala Leu Pro Arg Tyr Arg Leu Leu Arg Asp	
	570 575 580 585	
35	ctg ccg cgt ctg ctg cgg gcg ctg gac gcg cgg cct ttc gca gag gcc	1872
	Leu Pro Arg Leu Leu Arg Ala Leu Asp Ala Arg Pro Phe Ala Glu Ala	
	590 595 600	
40	acc agc tgg ggc cgc ctt ggg gcg cgg cag cgc agg cag agc cgc cta	1920
	Thr Ser Trp Gly Arg Leu Gly Ala Arg Gln Arg Arg Gln Ser Arg Leu	
	605 610 615	
	gag ctg tgc agc cgg ctc gaa cga gag gcc gcc cga ctt gca gac cta	1968
	Glu Leu Cys Ser Arg Leu Glu Arg Glu Ala Ala Arg Leu Ala Asp Leu	
	620 625 630	
45	ggt tgagcagagc tccaccgcag tcccggtgt ctgcggccgc t	2012
	Gly	

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50 MGSSRLAALLPLLLIIVIDLSDSAGIGFRHLPHWNTRCPLASHTEVLPISLAAPGGPSSPQSLGVCESGTVP  
AVCASICQVAQVFNGASSTSWCRNPKSLPHSSSIGDTRCQHLLRGSCCLVVTCLRRAITFPSPQTSPTRD  
FALKGPNLRIQRHGKVPFDWTHKGMEVGTGYNRRWVQLSGGPEFSFDLLPEARAIRVTISSGPEVSVRLCHQ  
WALECEELSSPYDVQKIVSGGHTVELPYEFLLPCLCIEASYLQEDTVRRKKCPFQSWPEAYGSDFWKSVHFT  
DYSQHTQMVMALTLRCPLKLEAALCQRHDWHTLCKDLNATARESDGWYVLEKVDLHPQLCFKVQPWFSFGN  
SSHVECPHQTSLSWNVSMDTQAQQLILHFSSRMHATFSAAWSLPGLGQDTLVPPVYTVSQVWRSDVQFAW  
KHLCPDVSRYRHLGLLILALLALLTLLGVVLALTCRRPQSGPGPARPVLLLHAADSEAQRRLVGALAEALLRA  
55 ALGGGRDIVDLWEGRHVARVGPLPWLWAARTRVAREQGTVLLLWSGADLRPVSGPDPRAAPLLALLHAAPR  
PLLLLAYFSRLCAKGDIPPLRLALPRYRLRLDLPRLLRALDARPF AEATSWGRLGARQRRQSRLELCSRLER  
EAARLADLG.

Reverse translation of primate, e.g., human, DCRS9 (SEQ ID NO: 18):

5 atgggnwsnw snmgnytnge ngcnytnytn ytnccnytny tnytnathgt nathgayytn 60  
 wsngaywsng cnggnathgg nttymgncay ytnccncayt ggaayacnmg ntgyccnytn 120  
 gcnwsncaya cngargtnyt nccnathwsn ytngcngcnc cnggnggncc nwsnwsnccn 180  
 10 carwsnytnng gngtntgyga rwsnggnacn gtncngcng tntgygcnws nathtgytgy 240  
 cargtngcnc argtnttyaa yggngcnwsn wsnacnwsnt ggtgymgnaa yccnaarwsn 300  
 15 ytnccncayw snwsnwsnat hggngayacn mgntgyarc ayytnytnmg nggnwsntgy 360  
 tgyytngtng tnacntgyyt nmgnmgngcn athacnttyc cnwsnccncc ncaracnwsn 420  
 ccnacnmng aytygcnyt naarggnccn ayytnmgna thcarmgna yggnaargtn 480  
 20 ttyccngayt ggacncayaa rggnatggar gtnggnacng gntayaaymg nmngtgggt 540  
 carytnwsng gnggncnga rttywsntty gayytnytn cngargcnmg ngcnathmgn 600  
 25 gtnacnathw snwsnggncc ngargtnwsn gtnmgnytn gycaycartg ggcnytngr 660  
 tgygargary tnwsnwsncc ntaygaygn caraarathg tnwsnggngg ncayacngtn 720  
 garytnccnt aygarttyt nytnccntgy ytntgyathg argcnwsnta yytnccarg 780  
 30 gayacngtnm gnmgnaaraa rtgyccntty carwsntggc cngargcnta yggnwsngay 840  
 ttytggaarw sngtnaytt yacngaytay wsnarcaya cncaratggt natggcnyn 900  
 35 acnytnmgnt gyccnytnaa rytngargcn gcnytnntgyc armgncayga ytggcayacn 960  
 ytntgyaarg ayytnccnaa ygcnacngcn mgngarwsng ayggntggta ygtnytngr 1020  
 aargtngayy tncayccna rytntgytty aargtnarc cntggttyws nttyggnaay 1080  
 40 wsnwsncayg tngartgycc ncaycaracn ggnwsnytna cnwsntggaa ygtnwsnatg 1140  
 gayacncarg cncarcaryt nathytnay ttywsnwsnm gnatgcaygc nacnttywsn 1200  
 45 gngcntggw snytnccngg nytnngncar gayacnytn tncncngnt ntayacngtn 1260  
 wsnargtn gmggnwsnga ygtncartty gentggaarc ayytnytn gycngaygn 1320  
 wsntaymgnc ayytnngny nytnathytn gcnytnytn cnytnytnac nytnytnngn 1380  
 50 gtngtnytn cnytnacntg ymgngnccn carwsnggnc cnggncngc nmgnccngtn 1440  
 ytnytnytn aygngcnga ywsngargcn carmgngny tngtnggngc nytnngngar 1500  
 55 ytnytnmgng cngcnytnng nggngngmgn gaygtnathg tngayytn ggarngmgn 1560  
 caygtngcnm gngtnggnc nytnccntgy ytntgggng cnmgnacnmg ngtnngmgn 1620  
 garcarggna cngtnytn nytntggnw gngcngayy tnmgnccngt nwsnggncn 1680

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 T

gayccnmgng	cngcncnyt	nytngenytn	ytncaygng	cncnmgnc	nytnytnytn	1740
ytngcntayt	tywsnmgny	ntgygcnaar	ggngayathc	cncncncny	nmgngcnytn	1800
cncmgntaym	gnytnytnmg	ngayytncn	mgnytnytnm	gngcnytnga	ygcnmgnccn	1860
ttygcngarg	cnacwsntg	gggnmgnytn	ggngcngmc	armgnmgnc	rwsnmgnytn	1920
garytntgyw	snmgnytnga	rmngngargcn	gcnmgnytn	cngayytngg	n	1971

Rodent, e.g., mouse, embodiment (see SEQ ID NO: 19 and 20). Predicted signal sequence indicated, but may vary by a few positions and depending upon cell type.

cagctccggg ccaggccctg ctgcccctctt gcagacagga aagacatggt ctctgcgccc															60
tgatcctaca gaagctc atg ggg agc ccc aga ctg gca gcc ttg ctc ctg															110
Met Gly Ser Pro Arg Leu Ala Ala Leu Leu Leu															
-20 -15															
tct ctc ccg cta ctg ctc atc ggc ctc gct gtg tct gct cgg gtt gcc															158
Ser Leu Pro Leu Leu Leu Ile Gly Leu Ala Val Ser Ala Arg Val Ala															
-10 -5 -1 1															
tgc ccc tgc ctg cgg agt tgg acc agc cac tgt ctc ctg gcc tac cgt															206
Cys Pro Cys Leu Arg Ser Trp Thr Ser His Cys Leu Leu Ala Tyr Arg															
5 10 15 20															
gtg gat aaa cgt ttt gct ggc ctt cag tgg ggc tgg ttc cct ctc ttg															254
Val Asp Lys Arg Phe Ala Gly Leu Gln Trp Gly Trp Phe Pro Leu Leu															
25 30 35															
gtg agg aaa tct aaa agt cct cct aaa ttt gaa gac tat tgg agg cac															302
Val Arg Lys Ser Lys Ser Pro Pro Lys Phe Glu Asp Tyr Trp Arg His															
40 45 50															
agg aca cca gca tcc ttc cag agg aag ctg cta ggc agc cct tcc ctg															350
Arg Thr Pro Ala Ser Phe Gln Arg Lys Leu Leu Gly Ser Pro Ser Leu															
55 60 65															
tct gag gaa agc cat cga att tcc atc ccc tcc tca gcc atc tcc cac															398
Ser Glu Glu Ser His Arg Ile Ser Ile Pro Ser Ser Ala Ile Ser His															
70 75 80															
aga ggc caa cgc acc aaa agg gcc cag cct tca gct gca gaa gga aga															446
Arg Gly Gln Arg Thr Lys Arg Ala Gln Pro Ser Ala Ala Glu Gly Arg															
85 90 95 100															
gaa cat ctc cct gaa gca ggg tca caa aag tgt gga gga cct gaa ttc															494
Glu His Leu Pro Glu Ala Gly Ser Gln Lys Cys Gly Gly Pro Glu Phe															
105 110 115															
tcc ttt gat ttg ctg ccc gag gtg cag gct gtt cgg gtg act att cct															542
Ser Phe Asp Leu Leu Pro Glu Val Gln Ala Val Arg Val Thr Ile Pro															
120 125 130															

atgggnwsnc	cnmgnythgc	ngcnytnytn	ytnwsnytn	cnytnytnyt	nathggnytn	60
gcngtnwsng	cnmgngtngc	ntgyccntgy	ytnmgnwsnt	ggacnwsnca	ytgyytnytn	120
gcntaymgng	tngayaarmg	nttygcnggn	ytncartggg	gntgggttycc	nytnytnngtn	180
mgnaarwsna	arwsnccncc	naarttygar	gaytaytggm	gncaymgnac	nccngcnwsn	240
ttycarmgna	arytnytnng	nwsnccnwsn	ytnwsngarg	arwsncaymg	nathwsnath	300
ccnwsnwsng	cnathwsnca	ymngngncar	mgnaacnaarm	gngcncarcc	nwsngcngcn	360
garggnmgng	arcayytnc	ngargcnggn	wsncaraart	gyggngngncc	ngarttywsn	420
ttygayytny	tncngargt	ncargcngtn	mgngtnacna	thccngcngg	nccnaargcn	480
mgngtnmgny	tntgytayca	rtgggcnytn	gartgygarg	ayytwnsnws	nccnttygay	540
acncaraara	thgtwnsngg	nggncayacn	gtngayytnc	cntaygartt	yytnytnccn	600
tgyatgtgya	thgargcnws	ntayytncar	gargayacng	tnmgngmgnaa	rwsngtnccn	660
wsnmgngcng	gnytnaaryt	natggcncar	acnwsnggnw	sncartaygc	nwsnytnacn	720
acnqcnwsn						729



Table 5: Nucleotide and polypeptide sequences of DNAX Cytokine Receptor Subunit like embodiments (DCRS10). Primate, e.g., human, embodiment (see SEQ ID NO: 22 and 23).

5	ttttgagcag aggcttccta ggctccgtag aaatttgcac acagcttcca cttcctgctt	60
	cagagcctgt tcttctactt acctgggccc ggagaaggtg gagggagacg agaagccgcc	120
10	gagagccgac taccctccgg gccagctctg tctgtccgtg gtggatctaa gaaactaga	179
	atg aac cga agc att cct gtg gag gtt gat gaa tca gaa cca tac cca	227
	Met Asn Arg Ser Ile Pro Val Glu Val Asp Glu Ser Glu Pro Tyr Pro	
	1 5 10 15	
15	agt cag ttg ctg aaa cca atc cca gaa tat tcc ccg gaa gag gaa tca	275
	Ser Gln Leu Leu Lys Pro Ile Pro Glu Tyr Ser Pro Glu Glu Glu Ser	
	20 25 30	
20	gaa cca cct gct cca aat ata agg aac atg gca ccc aac agc ttg tct	323
	Glu Pro Pro Ala Pro Asn Ile Arg Asn Met Ala Pro Asn Ser Leu Ser	
	35 40 45	
25	gca ccc aca atg ctt cac aat tcc tcc gga gac ttt tct caa gct cac	371
	Ala Pro Thr Met Leu His Asn Ser Ser Gly Asp Phe Ser Gln Ala His	
	50 55 60	
30	tca acc ctg aaa ctt gca aat cac cag cgg cct gta tcc cgg cag gtc	419
	Ser Thr Leu Lys Leu Ala Asn His Gln Arg Pro Val Ser Arg Gln Val	
	65 70 75 80	
35	acc tgc ctg cgc act caa gtt ctg gag gac agt gaa gac agt ttc tgc	467
	Thr Cys Leu Arg Thr Gln Val Leu Glu Asp Ser Glu Asp Ser Phe Cys	
	85 90 95	
40	agg aga cac cca ggc ctg ggc aaa gct ttc cct tct ggg tgc tct gca	515
	Arg Arg His Pro Gly Leu Gly Lys Ala Phe Pro Ser Gly Cys Ser Ala	
	100 105 110	
45	gtc agc gag cct gcg tct gag tct gtg gtt gga gcc ctc cct gca gag	563
	Val Ser Glu Pro Ala Ser Glu Ser Val Val Gly Ala Leu Pro Ala Glu	
	115 120 125	
50	cat cag ttt tca ttt atg gaa aaa cgt aat caa tgg ctg gta tct cag	611
	His Gln Phe Ser Phe Met Glu Lys Arg Asn Gln Trp Leu Val Ser Gln	
	130 135 140	
55	ctt tca gcg gct tct cct gac act ggc cat gac tca gac aaa tca gac	659
	Leu Ser Ala Ala Ser Pro Asp Thr Gly His Asp Ser Asp Lys Ser Asp	
	145 150 155 160	
60	caa agt tta cct aat gcc tca gca gac tcc ttg ggc ggt agc cag gag	707
	Gln Ser Leu Pro Asn Ala Ser Ala Asp Ser Leu Gly Gly Ser Gln Glu	
	165 170 175	
65	atg gtg caa cgg ccc cag cct cac agg aac cga gca ggc ctg gat ctg	755
	Met Val Gln Arg Pro Gln Pro His Arg Asn Arg Ala Gly Leu Asp Leu	
	180 185 190	

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09063810-052740  
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	cca acc ata gac acg gga tat gat tcc cag ccc cag gat gtc ctg ggc	803
	Pro Thr Ile Asp Thr Gly Tyr Asp Ser Gln Pro Gln Asp Val Leu Gly	
	195 200 205	
5	atc agg cag ctg gaa agg ccc ctg ccc ctc acc tcc gtg tgt tac ccc	851
	Ile Arg Gln Leu Glu Arg Pro Leu Pro Leu Thr Ser Val Cys Tyr Pro	
	210 215 220	
10	cag gac ctc ccc aga cct ctc agg tcc agg gag ttc cct cag ttt gaa	899
	Gln Asp Leu Pro Arg Pro Leu Arg Ser Arg Glu Phe Pro Gln Phe Glu	
	225 230 235 240	
15	cct cag agg tat cca gca tgt gca cag atg ctg cct ccc aat ctt tcc	947
	Pro Gln Arg Tyr Pro Ala Cys Ala Gln Met Leu Pro Pro Asn Leu Ser	
	245 250 255	
	cca cat gct cca tgg aac tat cat tac cat tgt cct gga agt ccc gat	995
	Pro His Ala Pro Trp Asn Tyr His Tyr His Cys Pro Gly Ser Pro Asp	
	260 265 270	
20	cac cag gtg cca tat ggc cat gac tac cct cga gca gcc tac cag caa	1043
	His Gln Val Pro Tyr Gly His Asp Tyr Pro Arg Ala Ala Tyr Gln Gln	
	275 280 285	
25	gtg atc cag ccg gct ctg cct ggg cag ccc ctg cct gga gcc agt gtg	1091
	Val Ile Gln Pro Ala Leu Pro Gly Gln Pro Leu Pro Gly Ala Ser Val	
	290 295 300	
30	aga ggc ctg cac cct gtg cag aag gtt atc ctg aat tat ccc agc ccc	1139
	Arg Gly Leu His Pro Val Gln Lys Val Ile Leu Asn Tyr Pro Ser Pro	
	305 310 315 320	
35	tgg gac caa gaa gag agg ccc gca cag aga gac tgc tcc ttt ccg ggg	1187
	Trp Asp Gln Glu Glu Arg Pro Ala Gln Arg Asp Cys Ser Phe Pro Gly	
	325 330 335	
40	ctt cca agg cac cag gac cag cca cat cac cag cca cct aat aga gct	1235
	Leu Pro Arg His Gln Asp Gln Pro His His Gln Pro Pro Asn Arg Ala	
	340 345 350	
	ggt gct cct ggg gag tcc ttg gag tgc cct gca gag ctg aga cca cag	1283
	Gly Ala Pro Gly Glu Ser Leu Glu Cys Pro Ala Glu Leu Arg Pro Gln	
	355 360 365	
45	gtt ccc cag cct ccg tcc cca gct gct gtg cct aga ccc cct agc aac	1331
	Val Pro Gln Pro Pro Ser Pro Ala Ala Val Pro Arg Pro Pro Ser Asn	
	370 375 380	
50	cct cca gcc aga gga act cta aaa aca agc aat ttg cca gaa gaa ttg	1379
	Pro Pro Ala Arg Gly Thr Leu Lys Thr Ser Asn Leu Pro Glu Glu Leu	
	385 390 395 400	
55	cgg aaa gtc ttt atc act tat tcg atg gac aca gct atg gag gtg gtg	1427
	Arg Lys Val Phe Ile Thr Tyr Ser Met Asp Thr Ala Met Glu Val Val	
	405 410 415	
	aaa ttc gtg aac ttt ttg ttg gta aat ggc ttc caa act gca att gac	1475
	Lys Phe Val Asn Phe Leu Leu Val Asn Gly Phe Gln Thr Ala Ile Asp	
	420 425 430	

20  
 25  
 30  
 35

	ata ttt gag gat aga atc cga ggc att gat atc att aaa tgg atg gag	1523
	Ile Phe Glu Asp Arg Ile Arg Gly Ile Asp Ile Ile Lys Trp Met Glu	
5	435 440 445	
	cgc tac ctt agg gat aag acc gtg atg ata atc gta gca atc agc ccc	1571
	Arg Tyr Leu Arg Asp Lys Thr Val Met Ile Ile Val Ala Ile Ser Pro	
	450 455 460	
10	aaa tac aaa cag gac gtg gaa ggc gct gag tcg cag ctg gac gag gat	1619
	Lys Tyr Lys Gln Asp Val Glu Gly Ala Glu Ser Gln Leu Asp Glu Asp	
	465 470 475 480	
15	gag cat ggc tta cat act aag tac att cat cga atg atg cag att gag	1667
	Glu His Gly Leu His Thr Lys Tyr Ile His Arg Met Met Gln Ile Glu	
	485 490 495	
20	ttc ata aaa caa gga agc atg aat ttc aga ttc atc cct gtg ctc ttc	1715
	Phe Ile Lys Gln Gly Ser Met Asn Phe Arg Phe Ile Pro Val Leu Phe	
	500 505 510	
25	cca aat gct aag aag gag cat gtg ccc acc tgg ctt cag aac act cat	1763
	Pro Asn Ala Lys Lys Glu His Val Pro Thr Trp Leu Gln Asn Thr His	
	515 520 525	
30	gtc tac agc tgg ccc aag aat aaa aaa aac atc ctg ctg cgg ctg ctg	1811
	Val Tyr Ser Trp Pro Lys Asn Lys Lys Asn Ile Leu Leu Arg Leu Leu	
	530 535 540	
35	aga gag gaa gag tat gtg gct cct cca cgg ggg cct ctg ccc acc ctt	1859
	Arg Glu Glu Glu Tyr Val Ala Pro Pro Arg Gly Pro Leu Pro Thr Leu	
	545 550 555 560	
	cag gtg gtt ccc ttg tgacaccggt catccccaga tctactgaggc caggccatgt	1914
	Gln Val Val Pro Leu	
	565	
40	ttggggcctt gttctgacag cattctggct gaggtctggtc ggtagcactc ctggctgggtt	1974
	tttttctgtt cctccccgag aggccctctg gccccagga aacctgttgt gcagagctct	2034
	tccccggaga cctccacaca ccctggcttt gaagtggagt ctgtgactgc tctgcattct	2094
45	ctgcttttaa aaaaaccatt gcaggtgcca gtgtcccata tgttcctcct gacagtttga	2154
	tgtgtccatt ctgggcctct cagtgccttag caagtagata atgtaagga tgtggcagca	2214
	aatggaaatg actacaaaca ctctcctatc aatcacttca ggctactttt atgagtttagc	2274
50	cagatgcttg tgtatcctca gaccaaaactg attcatgtac aaataataaa atgtttactc	2334
	ttttgtaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaa	2377

5

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**curry**

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## • Summary

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athgayatha thaartggat ggarmgntay ytnmgngaya aracngtnat gathathgtn 1380

gcnathwsnc cnaartayaa rcargaygtn gargngcng arwsncaryt ngaygargay 1440  
 garcayggny tncayacnaa rtayathcay mgnatgatgc arathgartt yathaarcay 1500  
 5 ggnwsnatga ayttymgntt yathccngtn ytnttyccna aygcnaaraa rgarcaygtn 1560  
 ccnacntggy tncaraayac ncaygtntay wsntggccna araayaaraa raayathytn 1620  
 10 ytnmgnytny tnmngngarga rgartaygtn gcncncncnm gnggncnny nccnacnytn 1680  
 cargtngtnc cnytn 1695

Rodent, e.g., mouse, embodiment (see SEQ ID NO: 25 and 26).

15 cag gac ctc cct ggg cct ctg agg tcc agg gaa ttg cca cct cag ttt 48  
 Gln Asp Leu Pro Gly Pro Leu Arg Ser Arg Glu Leu Pro Pro Gln Phe  
 1 5 10 15

20 gaa ctt gag agg tat cca atg aac gcc cag ctg ctg ccg ccc cat cct 96  
 Glu Leu Glu Arg Tyr Pro Met Asn Ala Gln Leu Leu Pro Pro His Pro  
 20 25 30

25 tcc cca cag gcc cca tgg aac tgt cag tac tac tgc ccc gga ggg ccc 144  
 Ser Pro Gln Ala Pro Trp Asn Cys Gln Tyr Tyr Cys Pro Gly Gly Pro  
 35 40 45

30 tac cac cac cag gtg cca cac ggc cat ggc tac cct cca gca gca gcc 192  
 Tyr His His Gln Val Pro His Gly His Gly Tyr Pro Pro Ala Ala Ala  
 50 55 60

35 tac cag caa gta ctc cag cct gct ctg cct ggg cag gtc ctt cct ggg 240  
 Tyr Gln Gln Val Leu Gln Pro Ala Leu Pro Gly Gln Val Leu Pro Gly  
 65 70 75 80

40 gca agg gca aga ggc cca cgc cct gtg cag aag gtc atc ctg aat gac 288  
 Ala Arg Ala Arg Gly Pro Arg Pro Val Gln Lys Val Ile Leu Asn Asp  
 85 90 95

45 tcc agc ccc caa gac caa gaa gag aga cct gca cag aga gac ttc tct 336  
 Ser Ser Pro Gln Asp Gln Glu Glu Arg Pro Ala Gln Arg Asp Phe Ser  
 100 105 110

50 ttc ccg agg ctc ccg agg gac cag ctc tac cgc cca cca tct aat gga 384  
 Phe Pro Arg Leu Pro Arg Asp Gln Leu Tyr Arg Pro Pro Ser Asn Gly  
 115 120 125

55 gtg gaa gcc cct gag gag tcc ttg gac ctt cct gca gag ctg aga cca 432  
 Val Glu Ala Pro Glu Glu Ser Leu Asp Leu Pro Ala Glu Leu Arg Pro  
 130 135 140

cat ggt ccc cag gct cca tcc cta gct gcc gtg cct aga ccc cct agc 480  
 His Gly Pro Gln Ala Pro Ser Leu Ala Ala Val Pro Arg Pro Pro Ser  
 145 150 155 160

aac ccc tta gcc cga gga act cta aga acc agc aat ttg cca gaa gaa 528  
 Asn Pro Leu Ala Arg Gly Thr Leu Arg Thr Ser Asn Leu Pro Glu Glu  
 165 170 175

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09067018 052304  
"09067018 052304"

tta cgg aaa gtc ttt atc act tat tct atg gac aca gcc atg gag gtg 576  
Leu Arg Lys Val Phe Ile Thr Tyr Ser Met Asp Thr Ala Met Glu Val  
180 185 190

5 gtg aaa ttt gtg aac ttt ctg ttg gtg aac ggc ttc caa act gcg att 624  
Val Lys Phe Val Asn Phe Leu Leu Val Asn Gly Phe Gln Thr Ala Ile  
195 200 205

10 gac ata ttt gag gat aga atc cgg ggt att gat atc att aaa tgg atg 672  
Asp Ile Phe Glu Asp Arg Ile Arg Gly Ile Asp Ile Ile Lys Trp Met  
210 215 220

15 gag cgc tat ctt cga gat aag aca gtg atg ata atc gta gca atc agc 720  
Glu Arg Tyr Leu Arg Asp Lys Thr Val Met Ile Ile Val Ala Ile Ser  
225 230 235 240

ccc aaa tac aaa cag gat gtg gaa ggc gct gag tcg cag ctg gac gag 768  
Pro Lys Tyr Lys Gln Asp Val Glu Gly Ala Glu Ser Gln Leu Asp Glu  
245 250 255

gac gag cat ggc tta cat act aag tac att cat cgg atg atg cag att 816  
Asp Glu His Gly Leu His Thr Lys Tyr Ile His Arg Met Met Gln Ile  
260 265 270

25 gag ttc ata agt cag gga agc atg aac ttc aga ttc atc cct gtg ctc 864  
Glu Phe Ile Ser Gln Gly Ser Met Asn Phe Arg Phe Ile Pro Val Leu  
275 280 285

30 ttc cca aat gcc aag aag gag cat gtg ccg acc tgg ctt cag aac act 912  
Phe Pro Asn Ala Lys Lys Glu His Val Pro Thr Trp Leu Gln Asn Thr  
290 295 300

35 cat gtt tac agc tgg ccc aag aat aag aaa aac atc ctg ctg cgg ctg 960  
His Val Tyr Ser Trp Pro Lys Asn Lys Lys Asn Ile Leu Leu Arg Leu  
305 310 315 320

ctc agg gag gaa gag tat gtg gct cct ccc cga ggc cct ctg ccc acc 1008  
Leu Arg Glu Glu Glu Tyr Val Ala Pro Pro Arg Gly Pro Leu Pro Thr  
325 330 335

40 ctt cag gtg gta ccc ttg tgacgatggc cactccagct cagtgccagc 1056  
Leu Gln Val Val Pro Leu  
340

45 ctgttctcac agcattcttc tagcggagct ggctggtggc acccaggccc tggaacacct 1116

cttctacaga gtctctgtgc tectgagtct gagttgtcct cgctgggctt ccagagcttc 1176

50 agtgccctgga tgctgcaggt gacagaaaca aacatctatg accacaaaaa ctctcatcac 1236

ttcagctact tttatgagtc ggtcagatgc tctgtgtcct tagaccagtc taaatcatgc 1296

tcaaataata aaatgattat tctttgt 1323

55 QDLPGPLRSRELPPQFELERYPMNAQLLPPHPSPQAPWNCQYYCPGGPYHHQVPHGHGYPPAAAYQQVLQPA  
LPGQVLPGARARGPRPVQKVILNDSSPDQEERPAQRDFSFPRLPRDQLYRPPSNGVEAPEESLDLPAELRP  
HGPQAPSLAAVPRPPSNPLARGTLRTSNLPEELRKVFITYSMDTAMEVVKFVNFLVNGFQTAIDIFEDRIR  
GIDI IKWMERYLRDKTVMIIVAISPKYKQDVEGAESQLDEDEHGLHTKYIHRMMQIEFISQGS MNFRFIPVL  
FPNAKKEHVPTWLQNTHVYSWPKNKKNILLRLLREEEYVAPPRGPLPTLQVVPL.

20 25 30 35

Table 6: Alignment of the cytoplasmic portions of various cytokine receptor subunits. The IL-17R\_Hu (SEQ ID NO: 28) is GenBank AAB99730.1(U58917), gi|7657230; the IL-17R\_Mu (SEQ ID NO: 29) is GenBank AAC52357.1(U31993), gi|6680411; the IL-17R\_Ce (SEQ ID NO: 30) is GenBank AAA811100.1(U39997), gi|1353171; and the DCRS6\_Ce (SEQ ID NO: 31) is EMBCAA90543.1(Z50177), gi|7503597. Of particular interest are motifs or features corresponding, in primate DCRS8 to: R/K at 339/340; D/E at 348/349; alpha-helical regions from H353-Q365, C370-S381, E389-H396, K410-D414, and D485-H495; beta sheet regions correspond to F400-V404 and F458-Y462; E at 431; E/D at 442/443; Y/F at 458; D/E at 468-470; Y/F at 481; and Q/R/F at 523.

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5	DCRS7_Mu	RTALLLHSADG-AGYERLVGALASALSQMP---	LRVAVDLWSRRE-LSAHGALAWFHHQR
	DCRS7_Hu	RAALLLYSADD-SGFERLVGALASALCQLP---	LRVAVDLWSRRE-LSAQGPVAVFHAQR
	IL-17R_Hu	RKVWIIYSADH-PLYVDVVLKFAQFLLTACG--	TEVALDLLEEQA-ISEAGVMTWVGRQK
	IL-17R_Mu	RKVWIVYSADH-PLYVEVVLKFAQFLITACG--	TEVALDLLEEQV-ISEVGVMTWVSRQK
	DCRS10	RKVFITYSMD----TAMEVVKFVNFLLVNG---	FQTAIDIFEDR--IRGIDI IKWMERYL
	DCRS10_Mu	RKVFITYSMD----TAMEVVKFVNFLLVNG---	FQTAIDIFEDR--IRGIDI IKWMERYL
	DCRS9_Hu	RPVLLLHAADS-EAQRRVLGALAE LLRAALGGGRDV	IVDLWEGRH-VARVGPLPWLWAAR
10	DCRS8_Hu	PKVFLCYSSKDGQNHMNVVQCFA YFLQDFCG--	CEVALDLWEDFS-LCREGQREWVIQKI
	IL-17R_Ce	VKVMIVYADDN-DLHTDCVKKLVENLRNCAS--	CDPVFDLEKLI--TAEIVPSRWLV DQI
	DCRS6_Hu	IKVLVVYPSEI--CFHHTICYFTEFLQNHCR--	SEVILEKWQKKK-IAEMGPVQWLATQK
	DCRS6_Ce	FKVMLVCPEVS-GRDED FMMRIADALKKS N--	NKVVC DRWFEDSKNAEENMLHWVYEQT
		. : . : *	
15	DCRS7_Mu	RRILQEGGVVILLFSPA AVAQCQ---	QWLQLQTVEP---GP---HDALAAWLS CVLPDFL
	DCRS7_Hu	RQTLQEGGVVLLFSPGAVALCS---	EWLQDGVSGPGAHP---HDAFRASLSCVLPDFL
	IL-17R_Hu	QEMVESNSKIIIVLCSRGT RAKWQALLGRGAP-	VRLRCDHGKPV-GDLFTAAMNMILPDFK
	IL-17R_Mu	QEMVESNSKIIILCSRG TQAKWKAILGWAEP	AVQLRCDHWKPA-GDLFTAAMNMILPDFK
20	DCRS10	R---DKTVMIIVAISP KYQDVE----	GAESQLDED-EHGL---HTKYIHRM-MQIEFIK
	DCRS10_Mu	R---DKTVMIIVAISP KYQDVE----	GAESQLDED-EHGL---HTKYIHRM-MQIEFIS
	DCRS9_Hu	TRVAREQGTVLLLWSGADLR PVS----	GPDP-RAAP-----LLA----LLHAAP
	DCRS8_Hu	H---ESQFIIIVVCSKGMKYFVD---	KNYKHKGGRGSGK---GELFLVAVSAIAEKL R
	IL-17R_Ce	S---SLKKFIIIVSDCAEKILD---	TEASETHQLVQARP--FADLFGPAMEMI IRDAT
	DCRS6_Hu	K---AADKVVFLLSNDVNSVCD---	GTCGKSEGSPSENS---QDLFPLAFNLFCSDLR
25	DCRS6_Ce	K---IAEKIIVFHSAYYHPRCG---	IYDVINNFFPCTDPR----LAHIALT---PEAQ
		... *	
30	DCRS7_Mu	QGRATGR-----YVG VYFDGLLHPDSVPSP	FRVAPLFSLP-SQLPAFLDALQ--GGCSTS
	DCRS7_Hu	QGRAPGS-----YVGACFDRLLHPDAVPAL	FRTVPVFTLP-SQLPDFLGALQ--QPRAPR
	IL-17R_Hu	RPACFGT-----YVVCYFSEVSCDGDVPDL	FGAAPRYPLM-DRFEEVYFRIQ--DLEMFO
	IL-17R_Mu	RPACFGT-----YVVCYFSGICSERDVPDL	FNITSRYPLM-DRFEEVYFRIQ--DLEMFE
	DCRS10	QGSMNFR-----FIPVLFPNAK-KEHVPTWL	QNTHVYSWP-KNKKNILLRLL-REEEYVA
	DCRS10_Mu	QGSMNFR-----FIPVLFPNAK-KEHVPTWL	QNTHVYSWP-KNKKNILLRLL-REEEYVA
	DCRS9_Hu	RPL-----LLLAYFSRLCAKGDIPPLRAL	PRYRL-RDLPRLLRALD--ARPF AE
35	DCRS8_Hu	QAKQSSAALS KFI AVYFDYSC-EGDVP	GILD LSTKYRLM-DNLPQLCSHLHSRDHGLQE
	IL-17R_Ce	HNFPEAR---KKYAVVRFNYSP---HVPPNL	AILNLPTFIPEQFAQLTAF LHN-VEHTER
	DCRS6_Hu	SQIHLHK-----YVVVFREID-TKDDYNAL	SVC PKYHLM-KDATAFCAELL--HVKQQ
	DCRS6_Ce	RSVPKEV---EYVLPRDQKLL--EDAFDITI	ADPLVIDIPIEDVAIPENVP--IH HESC
			:
40	DCRS7_Mu	AGRPADRVER-----VT----	QALRSALDSCTS-----
	DCRS7_Hu	SGRLQERAEQ-----VS----	RALQPALDSYFHPP-----
	IL-17R_Hu	PGRMHRVGELSGDNYLRS---	PGGRQLRAALDRFRDQVRC PDW
	IL-17R_Mu	PGRMHVREL TGDNYLQS---	PSGRQLKEAVLRFQEWQTQCPDW
45	DCRS10	P----PRGPL-----PTLQVVPL-----	
	DCRS10_Mu	P----PRGPL-----PTLQVVPL-----	
	DCRS9_Hu	ATSWGRLGAR-----QRRQSRLELC SR-----	
	DCRS8_Hu	PGQHTRQGS R-----RNYFRSKSGRSLYVA	ICNMHQFIDE EPDW
	IL-17R_Ce	ANVTQNISEA-----Q-----IHEWNL	CASRMMSFFVRNPW
50	DCRS6_Hu	VS---AGKR-----SQACHDGCCSL-----	
	DCRS6_Ce	DSIDSRNNSK-----THSTD SGVSSLSS-----	NS--
			:



~~INBA10~~ <sup>1910</sup> Table 6 shows comparison of the available sequences of primate, rodent, and various other receptors. Various conserved residues are aligned and indicated. The structurally homologous cytoplasmic domains most likely signal through pathways like IL-17, e.g., through NFkB. Similar to IL-1 signalling, it is likely that these receptors are involved in innate immunity and/or development.

<sup>5</sup> ~~INSA11~~ <sup>1911</sup> As used herein, the term DCRS shall be used to describe a protein comprising amino acid sequences shown in Tables 1-5, respectively. In many cases, a substantial ~~fragment thereof will be functionally or structurally equivalent, including, e.g., an~~ extracellular or intracellular domain. The invention also includes a protein variation of the respective DCRS allele whose sequence is provided, e.g., a mutein or soluble extracellular construct. Typically, such agonists or antagonists will exhibit less than about 10% sequence differences, and thus will often have between 1 and 11 substitutions, e.g., 2-, 3-, 5-, 7-fold, and others. It also encompasses allelic and other variants, e.g., natural polymorphic, of the protein described. Typically, it will bind to its corresponding biological ligand, perhaps in a dimerized state with an alpha receptor subunit, with high affinity, e.g., at least about 100 nM, usually better than about 30 nM, preferably better than about 10 nM, and more preferably at better than about 3 nM. The term shall also be used herein to refer to related naturally occurring forms, e.g., alleles, polymorphic variants, and metabolic variants of the mammalian protein. Preferred forms of the receptor complexes will bind the appropriate ligand with an affinity and selectivity appropriate for a ligand-receptor interaction.

<sup>15</sup> <sup>1912</sup> ~~INSA12~~ This invention also encompasses combinations of proteins or peptides having substantial amino acid sequence identity with an amino acid sequence in Tables 1-5. It will include sequence variants with relatively few residue substitutions, e.g., preferably less than about 3-5.

<sup>25</sup> A substantial polypeptide "fragment", or "segment", is a stretch of amino acid residues of at least about 8 amino acids, generally at least 10 amino acids, more generally at least 12 amino acids, often at least 14 amino acids, more often at least 16 amino acids, typically at least 18 amino acids, more typically at least 20 amino acids, usually at least 22 amino acids, more usually at least 24 amino acids, preferably at least 26 amino acids, more preferably at least 28 amino acids, and, in particularly preferred embodiments, at least about 30 or more amino acids. This includes, e.g., 40, 50, 60, 70, 85, 100, 115, 130, 150, and other lengths. Sequences of segments of different proteins can be compared to one another over appropriate length stretches, typically between conserved motifs. In many situations, fragments may exhibit functional properties of the intact subunits, e.g., the extracellular domain of the transmembrane receptor may retain the ligand binding features, and may be used to prepare a soluble receptor-like complex.

~~INSA13~~ ~~LA13~~ Amino acid sequence homology, or sequence identity, is determined by  
 optimizing residue matches. In some comparisons, gaps may be introduced, as required.  
 See, e.g., Needleham, et al., (1970) J. Mol. Biol. 48:443-453; Sankoff, et al., (1983)  
 chapter one in Time Warps, String Edits, and Macromolecules: The Theory and Practice  
 of Sequence Comparison, Addison-Wesley, Reading, MA; and software packages from  
 IntelliGenetics, Mountain View, CA; and the University of Wisconsin Genetics Computer  
 Group (GCG), Madison, WI; each of which is incorporated herein by reference. This  
 changes when considering conservative substitutions as matches. Conservative  
 substitutions typically include substitutions within the following groups: glycine,  
 alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine;  
 serine, threonine; lysine, arginine; and phenylalanine, tyrosine. Homologous amino acid  
 sequences are intended to include natural allelic and interspecies variations in the  
 cytokine sequence. Typical homologous proteins or peptides will have from 50-100%  
 homology (if gaps can be introduced), to 60-100% homology (if conservative  
 substitutions are included) with an amino acid sequence segment of, e.g., Table 3 or 4.  
 Homology measures will be at least about 70%, generally at least 76%, more generally at  
 least 81%, often at least 85%, more often at least 88%, typically at least 90%, more  
 typically at least 92%, usually at least 94%, more usually at least 95%, preferably at least  
 96%, and more preferably at least 97%, and in particularly preferred embodiments, at  
 least 98% or more. The degree of homology will vary with the length of the compared  
 segments. Homologous proteins or peptides, such as the allelic variants, will share most  
 biological activities with the embodiments described in Tables 1-5.

As used herein, the term "biological activity" is used to describe, without  
 limitation, effects on inflammatory responses, innate immunity, and/or morphogenic  
 development by cytokine-like ligands. For example, these receptors should mediate  
 phosphatase or phosphorylase activities, which activities are easily measured by standard  
 procedures. See, e.g., Hardie, et al. (eds. 1995) The Protein Kinase FactBook vols. I and  
 II, Academic Press, San Diego, CA; Hanks, et al. (1991) Meth. Enzymol. 200:38-62;  
 Hunter, et al. (1992) Cell 70:375-388; Lewin (1990) Cell 61:743-752; Pines, et al. (1991)  
Cold Spring Harbor Symp. Quant. Biol. 56:449-463; and Parker, et al. (1993) Nature  
 363:736-738. The receptors, or portions thereof, may be useful as phosphate labeling  
 enzymes to label general or specific substrates. The subunits may also be functional  
 immunogens to elicit recognizing antibodies, or antigens capable of binding antibodies.

The terms ligand, agonist, antagonist, and analog of, e.g., a DCRS8 or DCRS9,  
 include molecules that modulate the characteristic cellular responses to cytokine ligand  
 proteins, as well as molecules possessing the more standard structural binding  
 competition features of ligand-receptor interactions, e.g., where the receptor is a natural

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receptor or an antibody. The cellular responses likely are typically mediated through receptor tyrosine kinase pathways.

Also, a ligand is a molecule which serves either as a natural ligand to which said receptor, or an analog thereof, binds, or a molecule which is a functional analog of the natural ligand. The functional analog may be a ligand with structural modifications, or may be a wholly unrelated molecule which has a molecular shape which interacts with the appropriate ligand binding determinants. The ligands may serve as agonists or antagonists, see, e.g., Goodman, et al. (eds. 1990) Goodman & Gilman's: The Pharmacological Bases of Therapeutics, Pergamon Press, New York.

Rational drug design may also be based upon structural studies of the molecular shapes of a receptor or antibody and other effectors or ligands. See, e.g., Herz, et al. (1997) J. Recept. Signal Transduct. Res. 17:671-776; and Chaiken, et al. (1996) Trends Biotechnol. 14:369-375. Effectors may be other proteins which mediate other functions in response to ligand binding, or other proteins which normally interact with the receptor. One means for determining which sites interact with specific other proteins is a physical structure determination, e.g., x-ray crystallography or 2 dimensional NMR techniques. These will provide guidance as to which amino acid residues form molecular contact regions. For a detailed description of protein structural determination, see, e.g., Blundell and Johnson (1976) Protein Crystallography, Academic Press, New York, which is hereby incorporated herein by reference.

## II. Activities

The cytokine receptor-like proteins will have a number of different biological activities, e.g., modulating cell proliferation, or in phosphate metabolism, being added to or removed from specific substrates, typically proteins. Such will generally result in modulation of an inflammatory function, other innate immunity response, or a morphological effect. The subunit will probably have a specific low affinity binding to the ligand.

The DCRS8 and DCRS9 have characteristic motifs of receptors signaling through the JAK pathway. See, e.g., Ihle, et al. (1997) Stem Cells 15(suppl. 1):105-111; Silvennoinen, et al. (1997) APMIS 105:497-509; Levy (1997) Cytokine Growth Factor Review 8:81-90; Winston and Hunter (1996) Current Biol. 6:668-671; Barrett (1996) Baillieres Clin. Gastroenterol. 10:1-15; and Briscoe, et al. (1996) Philos. Trans. R. Soc. Lond. B. Biol. Sci. 351:167-171.

The biological activities of the cytokine receptor subunits will be related to addition or removal of phosphate moieties to substrates, typically in a specific manner, but occasionally in a non specific manner. Substrates may be identified, or conditions for

enzymatic activity may be assayed by standard methods, e.g., as described in Hardie, et al. (eds. 1995) The Protein Kinase FactBook vols. I and II, Academic Press, San Diego, CA; Hanks, et al. (1991) Meth. Enzymol. 200:38-62; Hunter, et al. (1992) Cell 70:375-388; Lewin (1990) Cell 61:743-752; Pines, et al. (1991) Cold Spring Harbor Symp. Quant. Biol. 56:449-463; and Parker, et al. (1993) Nature 363:736-738.

The receptor subunits may combine to form functional complexes, e.g., which may be useful for binding ligand or preparing antibodies. These will have substantial diagnostic uses, including detection or quantitation.

### III. Nucleic Acids

~~This invention contemplates use of isolated nucleic acid or fragments, e.g., which encode these or closely related proteins, or fragments thereof, e.g., to encode a corresponding polypeptide, preferably one which is biologically active. In addition, this invention covers isolated or recombinant DNAs which encode combinations of such proteins or polypeptides having characteristic sequences, e.g., of the DCRSSs. Typically, the nucleic acid is capable of hybridizing, under appropriate conditions, with a nucleic acid sequence segment shown in Tables 1-5, but preferably not with a corresponding segment of other receptors described in Table 6. Said biologically active protein or polypeptide can be a full length protein, or fragment, and will typically have a segment of amino acid sequence highly homologous, e.g., exhibiting significant stretches of identity, to one shown in Tables 1-5. Further, this invention covers the use of isolated or recombinant nucleic acid, or fragments thereof, which encode proteins having fragments which are equivalent to the DCRS8 or DCRS9 proteins. The isolated nucleic acids can have the respective regulatory sequences in the 5' and 3' flanks, e.g., promoters, enhancers, poly-A addition signals, and others from the natural gene. Combinations, as described, are also provided.~~

An "isolated" nucleic acid is a nucleic acid, e.g., an RNA, DNA, or a mixed polymer, which is substantially pure, e.g., separated from other components which naturally accompany a native sequence, such as ribosomes, polymerases, and flanking genomic sequences from the originating species. The term embraces a nucleic acid sequence which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates, which are thereby distinguishable from naturally occurring compositions, and chemically synthesized analogs or analogs biologically synthesized by heterologous systems. A substantially pure molecule includes isolated forms of the molecule, either completely or substantially pure.

An isolated nucleic acid will generally be a homogeneous composition of molecules, but will, in some embodiments, contain heterogeneity, preferably minor. This

heterogeneity is typically found at the polymer ends or portions not critical to a desired biological function or activity.

5 A "recombinant" nucleic acid is typically defined either by its method of production or its structure. In reference to its method of production, e.g., a product made by a process, the process is use of recombinant nucleic acid techniques, e.g., involving human intervention in the nucleotide sequence. Typically this intervention involves in vitro manipulation, although under certain circumstances it may involve more classical animal breeding techniques. Alternatively, it can be a nucleic acid made by generating a sequence comprising fusion of two fragments which are not naturally contiguous to each other, but is meant to exclude products of nature, e.g., naturally occurring mutants as found in their natural state. Thus, for example, products made by transforming cells with an unnaturally occurring vector is encompassed, as are nucleic acids comprising sequence derived using any synthetic oligonucleotide process. Such a process is often done to replace a codon with a redundant codon encoding the same or a conservative amino acid, while typically introducing or removing a restriction enzyme sequence recognition site. Alternatively, the process is performed to join together nucleic acid segments of desired functions to generate a single genetic entity comprising a desired combination of functions not found in the commonly available natural forms, e.g., encoding a fusion protein. Restriction enzyme recognition sites are often the target of such artificial manipulations, but other site specific targets, e.g., promoters, DNA replication sites, regulation sequences, control sequences, or other useful features may be incorporated by design. A similar concept is intended for a recombinant, e.g., fusion, polypeptide. This will include a dimeric repeat. Specifically included are synthetic nucleic acids which, by genetic code redundancy, encode equivalent polypeptides to fragments of DCRSs and fusions of sequences from various different related molecules, e.g., other cytokine receptor family members.

10 20 30 35 A "fragment" in a nucleic acid context is a contiguous segment of at least about 17 nucleotides, generally at least 21 nucleotides, more generally at least 25 nucleotides, ordinarily at least 30 nucleotides, more ordinarily at least 35 nucleotides, often at least 39 nucleotides, more often at least 45 nucleotides, typically at least 50 nucleotides, more typically at least 55 nucleotides, usually at least 60 nucleotides, more usually at least 66 nucleotides, preferably at least 72 nucleotides, more preferably at least 79 nucleotides, and in particularly preferred embodiments will be at least 85 or more nucleotides. Typically, fragments of different genetic sequences can be compared to one another over appropriate length stretches, particularly defined segments such as the domains described below.

A nucleic acid which codes for the DCRS8 or DCRS9 will be particularly useful to identify genes, mRNA, and cDNA species which code for itself or closely related proteins, as well as DNAs which code for polymorphic, allelic, or other genetic variants, e.g., from different individuals or related species. Preferred probes for such screens are those regions of the interleukin which are conserved between different polymorphic variants or which contain nucleotides which lack specificity, and will preferably be full length or nearly so. In other situations, polymorphic variant specific sequences will be more useful.

This invention further covers recombinant nucleic acid molecules and fragments having a nucleic acid sequence identical to or highly homologous to the isolated DNA set forth herein. In particular, the sequences will often be operably linked to DNA segments which control transcription, translation, and DNA replication. These additional segments typically assist in expression of the desired nucleic acid segment.

Homologous, or highly identical, nucleic acid sequences, when compared to one another, e.g., DCRS8 sequences, exhibit significant similarity. The standards for homology in nucleic acids are either measures for homology generally used in the art by sequence comparison or based upon hybridization conditions. Comparative hybridization conditions are described in greater detail below.

~~1915 Substantial identity in the nucleic acid sequence comparison context means either that the segments, or their complementary strands, when compared, are identical when optimally aligned, with appropriate nucleotide insertions or deletions, in at least about 60% of the nucleotides, generally at least 66%, ordinarily at least 71%, often at least 76%, more often at least 80%, usually at least 84%, more usually at least 88%, typically at least 91%, more typically at least about 93%, preferably at least about 95%, more preferably at least about 96 to 98% or more, and in particular embodiments, as high at about 99% or more of the nucleotides, including, e.g., segments encoding structural domains such as the segments described below. Alternatively, substantial identity will exist when the segments will hybridize under selective hybridization conditions, to a strand or its complement, typically using a sequence derived from Tables 1-5. Typically, selective hybridization will occur when there is at least about 55% homology over a stretch of at least about 14 nucleotides, more typically at least about 65%, preferably at least about 75%, and more preferably at least about 90%. See, Kanehisa (1984) Nucl. Acids Res. 12:203-213, which is incorporated herein by reference. The length of homology comparison, as described, may be over longer stretches, and in certain embodiments will be over a stretch of at least about 17 nucleotides, generally at least about 20 nucleotides, ordinarily at least about 24 nucleotides, usually at least about 28 nucleotides, typically at least about 32 nucleotides, more typically at least about 40 nucleotides, preferably at least~~

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a ~~about 50 nucleotides, and more preferably at least about 75 to 100 or more nucleotides.~~  
~~This includes, e.g., 125, 150, 175, 200, 225, 246, 273, and other lengths.~~

Stringent conditions, in referring to homology in the hybridization context, will be stringent combined conditions of salt, temperature, organic solvents, and other parameters typically controlled in hybridization reactions. Stringent temperature conditions will usually include temperatures in excess of about 30 C, more usually in excess of about 37 C, typically in excess of about 45 C, more typically in excess of about 55 C, preferably in excess of about 65 C, and more preferably in excess of about 70 C. Stringent salt conditions will ordinarily be less than about 500 mM, usually less than about 400 mM, more usually less than about 300 mM, typically less than about 200 mM, preferably less than about 100 mM, and more preferably less than about 80 mM, even down to less than about 20 mM. However, the combination of parameters is much more important than the measure of any single parameter. See, e.g., Wetmur and Davidson (1968) J. Mol. Biol. 31:349-370, which is hereby incorporated herein by reference.

The isolated DNA can be readily modified by nucleotide substitutions, nucleotide deletions, nucleotide insertions, and inversions of nucleotide stretches. These modifications result in novel DNA sequences which encode this protein or its derivatives. These modified sequences can be used to produce mutant proteins (muteins) or to enhance the expression of variant species. Enhanced expression may involve gene amplification, increased transcription, increased translation, and other mechanisms. Such mutant DCRS8-like derivatives include predetermined or site-specific mutations of the protein or its fragments, including silent mutations using genetic code degeneracy. "Mutant DCRS8" as used herein encompasses a polypeptide otherwise falling within the homology definition of the DCRS8 as set forth above, but having an amino acid sequence which differs from that of other cytokine receptor-like proteins as found in nature, whether by way of deletion, substitution, or insertion. In particular, "site specific mutant DCRS8" encompasses a protein having substantial sequence identity with a protein of Table 3, and typically shares most of the biological activities or effects of the forms disclosed herein.

Although site specific mutation sites are predetermined, mutants need not be site specific. Mammalian DCRS8 mutagenesis can be achieved by making amino acid insertions or deletions in the gene, coupled with expression. Substitutions, deletions, insertions, or many combinations may be generated to arrive at a final construct. Insertions include amino- or carboxy- terminal fusions. Random mutagenesis can be conducted at a target codon and the expressed mammalian DCRS mutants can then be screened for the desired activity, providing some aspect of a structure-activity relationship. Methods for making substitution mutations at predetermined sites in DNA

having a known sequence are well known in the art, e.g., by M13 primer mutagenesis. See also Sambrook, et al. (1989) and Ausubel, et al. (1987 and periodic Supplements).

The mutations in the DNA normally should not place coding sequences out of reading frames and preferably will not create complementary regions that could hybridize to produce secondary mRNA structure such as loops or hairpins.

The phosphoramidite method described by Beaucage and Carruthers (1981) Tetra. Letts. 22:1859-1862, will produce suitable synthetic DNA fragments. A double stranded fragment will often be obtained either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

Polymerase chain reaction (PCR) techniques can often be applied in mutagenesis. Alternatively, mutagenesis primers are commonly used methods for generating defined mutations at predetermined sites. See, e.g., Innis, et al. (eds. 1990) PCR Protocols: A Guide to Methods and Applications Academic Press, San Diego, CA; and Dieffenbach and Dveksler (1995; eds.) PCR Primer: A Laboratory Manual Cold Spring Harbor Press, CSH, NY.

Certain embodiments of the invention are directed to combination compositions comprising the receptor or ligand sequences described. In other embodiments, functional portions of the sequences may be joined to encode fusion proteins. In other forms, variants of the described sequences may be substituted.

#### IV. Proteins, Peptides

~~As described above, the present invention encompasses primate DCRS6-10, e.g., whose sequences are disclosed in Tables 1-5, and described above. Allelic and other variants are also contemplated, including, e.g., fusion proteins combining portions of such sequences with others, including, e.g., epitope tags and functional domains.~~

The present invention also provides recombinant proteins, e.g., heterologous fusion proteins using segments from these primate or rodent proteins. A heterologous fusion protein is a fusion of proteins or segments which are naturally not normally fused in the same manner. Thus, the fusion product of, e.g., a DCRS8 with another cytokine receptor is a continuous protein molecule having sequences fused in a typical peptide linkage, typically made as a single translation product and exhibiting properties, e.g., sequence or antigenicity, derived from each source peptide. A similar concept applies to heterologous nucleic acid sequences. Combinations of various designated proteins into complexes are also provided.

In addition, new constructs may be made from combining similar functional or structural domains from other related proteins, e.g., cytokine receptors or Toll-like

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receptors, including species variants. For example, ligand-binding or other segments may be "swapped" between different new fusion polypeptides or fragments. See, e.g., Cunningham, et al. (1989) Science 243:1330-1336; and O'Dowd, et al. (1988) J. Biol. Chem. 263:15985-15992, each of which is incorporated herein by reference. Thus, new chimeric polypeptides exhibiting new combinations of specificities will result from the functional linkage of receptor-binding specificities. For example, the ligand binding domains from other related receptor molecules may be added or substituted for other domains of this or related proteins. The resulting protein will often have hybrid function and properties. For example, a fusion protein may include a targeting domain which may serve to provide sequestering of the fusion protein to a particular subcellular organelle.

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~~Ag17~~ ~~Candidate fusion partners and sequences can be selected from various sequence data bases, e.g., GenBank, c/o IntelliGenetics, Mountain View, CA; and BCG, University of Wisconsin Biotechnology Computing Group, Madison, WI, which are each incorporated herein by reference. In particular, combinations of polypeptide sequences provided in Tables 1-5 are particularly preferred. Variant forms of the proteins may be substituted in the described combinations.~~

The present invention particularly provides muteins which bind cytokine-like ligands, and/or which are affected in signal transduction. Structural alignment of human DCRSs with other members of the cytokine receptor family show conserved features/residues. See Table 6. Alignment of the human DCRS8 sequence with other members of the cytokine receptor family indicates various structural and functionally shared features. See also, Bazan, et al. (1996) Nature 379:591; Lodi, et al. (1994) Science 263:1762-1766; Sayle and Milner-White (1995) TIBS 20:374-376; and Gronenberg, et al. (1991) Protein Engineering 4:263-269.

Substitutions with either mouse sequences or human sequences are particularly preferred. Conversely, conservative substitutions away from the ligand binding interaction regions will probably preserve most signaling activities; and conservative substitutions away from the intracellular domains will probably preserve most ligand binding properties.

"Derivatives" of the primate DCRS8 include amino acid sequence mutants, glycosylation variants, metabolic derivatives and covalent or aggregative conjugates with other chemical moieties. Covalent derivatives can be prepared by linkage of functionalities to groups which are found in the DCRS8 amino acid side chains or at the N- or C- termini, e.g., by means which are well known in the art. These derivatives can include, without limitation, aliphatic esters or amides of the carboxyl terminus, or of residues containing carboxyl side chains, O-acyl derivatives of hydroxyl group-containing residues, and N-acyl derivatives of the amino terminal amino acid or amino-group

containing residues, e.g., lysine or arginine. Acyl groups are selected from the group of alkyl-moieties, including C3 to C18 normal alkyl, thereby forming alkanoyl aroyl species.

In particular, glycosylation alterations are included, e.g., made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing, or in further processing steps. Particularly preferred means for accomplishing this are by exposing the polypeptide to glycosylating enzymes derived from cells which normally provide such processing, e.g., mammalian glycosylation enzymes. Deglycosylation enzymes are also contemplated. Also embraced are versions of the same primary amino acid sequence which have other minor modifications, including phosphorylated amino acid residues, e.g., phosphotyrosine, phosphoserine, or phosphothreonine.

A major group of derivatives are covalent conjugates of the receptors or fragments thereof with other proteins of polypeptides. These derivatives can be synthesized in recombinant culture such as N- or C-terminal fusions or by the use of agents known in the art for their usefulness in cross-linking proteins through reactive side groups. Preferred derivatization sites with cross-linking agents are at free amino groups, carbohydrate moieties, and cysteine residues.

Fusion polypeptides between the receptors and other homologous or heterologous proteins are also provided. Homologous polypeptides may be fusions between different receptors, resulting in, for instance, a hybrid protein exhibiting binding specificity for multiple different cytokine ligands, or a receptor which may have broadened or weakened specificity of substrate effect. Likewise, heterologous fusions may be constructed which would exhibit a combination of properties or activities of the derivative proteins. Typical examples are fusions of a reporter polypeptide, e.g., luciferase, with a segment or domain of a receptor, e.g., a ligand-binding segment, so that the presence or location of a desired ligand may be easily determined. See, e.g., Dull, et al., U.S. Patent No. 4,859,609, which is hereby incorporated herein by reference. Other gene fusion partners include glutathione-S-transferase (GST), bacterial  $\beta$ -galactosidase, trpE, Protein A,  $\beta$ -lactamase, alpha amylase, alcohol dehydrogenase, and yeast alpha mating factor. See, e.g., Godowski, et al. (1988) Science 241:812-816. Labeled proteins will often be substituted in the described combinations of proteins.

The phosphoramidite method described by Beaucage and Carruthers (1981) Tetra. Letts. 22:1859-1862, will produce suitable synthetic DNA fragments. A double stranded fragment will often be obtained either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

Such polypeptides may also have amino acid residues which have been chemically modified by phosphorylation, sulfonation, biotinylation, or the addition or removal of

other moieties, particularly those which have molecular shapes similar to phosphate groups. In some embodiments, the modifications will be useful labeling reagents, or serve as purification targets, e.g., affinity ligands.

Fusion proteins will typically be made by either recombinant nucleic acid methods or by synthetic polypeptide methods. Techniques for nucleic acid manipulation and expression are described generally, for example, in Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual (2d ed.), Vols. 1-3, Cold Spring Harbor Laboratory, and Ausubel, et al. (eds. 1987 and periodic supplements) Current Protocols in Molecular Biology, Greene/Wiley, New York, which are each incorporated herein by reference. Techniques for synthesis of polypeptides are described, for example, in Merrifield (1963) J. Amer. Chem. Soc. 85:2149-2156; Merrifield (1986) Science 232: 341-347; and Atherton, et al. (1989) Solid Phase Peptide Synthesis: A Practical Approach, IRL Press, Oxford; each of which is incorporated herein by reference. See also Dawson, et al. (1994) Science 266:776-779 for methods to make larger polypeptides.

This invention also contemplates the use of derivatives of a DCRS8 other than variations in amino acid sequence or glycosylation. Such derivatives may involve covalent or aggregative association with chemical moieties. These derivatives generally fall into three classes: (1) salts, (2) side chain and terminal residue covalent modifications, and (3) adsorption complexes, for example with cell membranes. Such covalent or aggregative derivatives are useful as immunogens, as reagents in immunoassays, or in purification methods such as for affinity purification of a receptor or other binding molecule, e.g., an antibody. For example, a cytokine ligand can be immobilized by covalent bonding to a solid support such as cyanogen bromide-activated Sepharose, by methods which are well known in the art, or adsorbed onto polyolefin surfaces, with or without glutaraldehyde cross-linking, for use in the assay or purification of a cytokine receptor, antibodies, or other similar molecules. The ligand can also be labeled with a detectable group, for example radioiodinated by the chloramine T procedure, covalently bound to rare earth chelates, or conjugated to another fluorescent moiety for use in diagnostic assays.

30 ~~A combination, e.g., including a DCRS8, of this invention can be used as an immunogen for the production of antisera or antibodies specific, e.g., capable of distinguishing between other cytokine receptor family members, for the combinations described. The complexes can be used to screen monoclonal antibodies or antigen-binding fragments prepared by immunization with various forms of impure preparations containing the protein. In particular, the term "antibodies" also encompasses antigen binding fragments of natural antibodies, e.g., Fab, Fab2, Fv, etc. The purified DCRS8 can also be used as a reagent to detect antibodies generated in response to the presence of~~

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<sup>a</sup> ~~elevated levels of expression, or immunological disorders which lead to antibody~~  
~~production to the endogenous receptor.~~ Additionally, DCRS8 fragments may also serve  
 as immunogens to produce the antibodies of the present invention, as described  
 immediately below. For example, this invention contemplates antibodies having binding  
 5 affinity to or being raised against the amino acid sequences shown in Tables 1-5,  
 fragments thereof, or various homologous peptides. In particular, this invention  
<sup>a</sup> ~~contemplates antibodies having binding affinity to, or having been raised against, specific~~  
~~fragments which are predicted to be, or actually are, exposed at the exterior protein~~  
~~surface of the native DCRS8 or DCRS9. Complexes of combinations of proteins will~~  
 10 ~~also be useful, and antibody preparations thereto can be made.~~

The blocking of physiological response to the receptor ligands may result from the  
 inhibition of binding of the ligand to the receptor, likely through competitive inhibition.  
 Thus, in vitro assays of the present invention will often use antibodies or antigen binding  
 segments of these antibodies, or fragments attached to solid phase substrates. These  
 15 assays will also allow for the diagnostic determination of the effects of either ligand  
 binding region mutations and modifications, or other mutations and modifications, e.g.,  
 which affect signaling or enzymatic function.

This invention also contemplates the use of competitive drug screening assays,  
 e.g., where neutralizing antibodies to the receptor complexes or fragments compete with a  
 20 test compound for binding to a ligand or other antibody. In this manner, the neutralizing  
 antibodies or fragments can be used to detect the presence of a polypeptide which shares  
 one or more binding sites to a receptor and can also be used to occupy binding sites on a  
 receptor that might otherwise bind a ligand.

## 25 V. Making Nucleic Acids and Protein

<sup>19</sup> ~~DNA which encodes the protein or fragments thereof can be obtained by chemical~~  
<sup>INSA19</sup> ~~synthesis, screening cDNA libraries, or by screening genomic libraries prepared from a~~  
~~wide variety of cell lines or tissue samples. Natural sequences can be isolated using~~  
~~standard methods and the sequences provided herein, e.g., in Tables 1-5. Other species~~  
 30 ~~counterparts can be identified by hybridization techniques, or by various PCR techniques,~~  
~~combined with or by searching in sequence databases, e.g., GenBank.~~

This DNA can be expressed in a wide variety of host cells for the synthesis of a  
 full-length receptor or fragments which can in turn, for example, be used to generate  
 polyclonal or monoclonal antibodies; for binding studies; for construction and expression  
 35 of modified ligand binding or kinase/phosphatase domains; and for structure/function  
 studies. Variants or fragments can be expressed in host cells that are transformed or  
 transfected with appropriate expression vectors. These molecules can be substantially

free of protein or cellular contaminants, other than those derived from the recombinant host, and therefore are particularly useful in pharmaceutical compositions when combined with a pharmaceutically acceptable carrier and/or diluent. The protein, or portions thereof, may be expressed as fusions with other proteins. Combinations of the described proteins, or nucleic acids encoding them, are particularly interesting.

Expression vectors are typically self-replicating DNA or RNA constructs containing the desired receptor gene or its fragments, usually operably linked to suitable genetic control elements that are recognized in a suitable host cell. These control elements are capable of effecting expression within a suitable host. The multiple genes may be coordinately expressed, and may be on a polycistronic message. The specific type of control elements necessary to effect expression will depend upon the eventual host cell used. Generally, the genetic control elements can include a prokaryotic promoter system or a eukaryotic promoter expression control system, and typically include a transcriptional promoter, an optional operator to control the onset of transcription, transcription enhancers to elevate the level of mRNA expression, a sequence that encodes a suitable ribosome binding site, and sequences that terminate transcription and translation. Expression vectors also usually contain an origin of replication that allows the vector to replicate independently of the host cell.

The vectors of this invention include those which contain DNA which encodes a combination of proteins, as described, or a biologically active equivalent polypeptide. The DNA can be under the control of a viral promoter and can encode a selection marker. This invention further contemplates use of such expression vectors which are capable of expressing eukaryotic cDNAs coding for such proteins in a prokaryotic or eukaryotic host, where the vector is compatible with the host and where the eukaryotic cDNAs are inserted into the vector such that growth of the host containing the vector expresses the cDNAs in question. Usually, expression vectors are designed for stable replication in their host cells or for amplification to greatly increase the total number of copies of the desirable gene per cell. It is not always necessary to require that an expression vector replicate in a host cell, e.g., it is possible to effect transient expression of the protein or its fragments in various hosts using vectors that do not contain a replication origin that is recognized by the host cell. It is also possible to use vectors that cause integration of the protein encoding portions into the host DNA by recombination.

Vectors, as used herein, comprise plasmids, viruses, bacteriophage, integratable DNA fragments, and other vehicles which enable the integration of DNA fragments into the genome of the host. Expression vectors are specialized vectors which contain genetic control elements that effect expression of operably linked genes. Plasmids are the most commonly used form of vector but all other forms of vectors which serve an equivalent

function and which are, or become, known in the art are suitable for use herein. See, e.g., Pouwels, et al. (1985 and Supplements) Cloning Vectors: A Laboratory Manual, Elsevier, N.Y., and Rodriguez, et al. (eds. 1988) Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Butterworth, Boston, which are incorporated herein by reference.

5 Transformed cells are cells, preferably mammalian, that have been transformed or transfected with vectors constructed using recombinant DNA techniques. Transformed host cells usually express the desired proteins, but for purposes of cloning, amplifying, and manipulating its DNA, do not need to express the subject proteins. This invention further contemplates culturing transformed cells in a nutrient medium, thus permitting the  
10 proteins to accumulate. The proteins can be recovered, either from the culture or, in certain instances, from the culture medium.

For purposes of this invention, nucleic sequences are operably linked when they are functionally related to each other. For example, DNA for a presequence or secretory leader is operably linked to a polypeptide if it is expressed as a preprotein or participates  
15 in directing the polypeptide to the cell membrane or in secretion of the polypeptide. A promoter is operably linked to a coding sequence if it controls the transcription of the polypeptide; a ribosome binding site is operably linked to a coding sequence if it is positioned to permit translation. Usually, operably linked means contiguous and in reading frame, however, certain genetic elements such as repressor genes are not  
20 contiguously linked but still bind to operator sequences that in turn control expression.

Suitable host cells include prokaryotes, lower eukaryotes, and higher eukaryotes. Prokaryotes include both gram negative and gram positive organisms, e.g., E. coli and B. subtilis. Lower eukaryotes include yeasts, e.g., S. cerevisiae and Pichia, and species of the genus Dictyostelium. Higher eukaryotes include established tissue culture cell lines  
25 from animal cells, both of non-mammalian origin, e.g., insect cells, and birds, and of mammalian origin, e.g., human, primates, and rodents.

Prokaryotic host-vector systems include a wide variety of vectors for many different species. As used herein, E. coli and its vectors will be used generically to include equivalent vectors used in other prokaryotes. A representative vector for  
30 amplifying DNA is pBR322 or many of its derivatives. Vectors that can be used to express the receptor or its fragments include, but are not limited to, such vectors as those containing the lac promoter (pUC-series); trp promoter (pBR322-trp); Ipp promoter (the pIN-series); lambda-pP or pR promoters (pOTS); or hybrid promoters such as ptac (pDR540). See Brosius, et al. (1988) "Expression Vectors Employing Lambda-, trp-, lac-, and Ipp-derived Promoters", in Vectors: A Survey of Molecular Cloning Vectors and  
35 Their Uses, (eds. Rodriguez and Denhardt), Butterworth, Boston, Chapter 10, pp. 205-236, which is incorporated herein by reference.

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Lower eukaryotes, e.g., yeasts and Dictyostelium, may be transformed with DCRS8 sequence containing vectors. For purposes of this invention, the most common lower eukaryotic host is the baker's yeast, Saccharomyces cerevisiae. It will be used to generically represent lower eukaryotes although a number of other strains and species are also available. Yeast vectors typically consist of a replication origin (unless of the integrating type), a selection gene, a promoter, DNA encoding the receptor or its fragments, and sequences for translation termination, polyadenylation, and transcription termination. Suitable expression vectors for yeast include such constitutive promoters as 3-phosphoglycerate kinase and various other glycolytic enzyme gene promoters or such inducible promoters as the alcohol dehydrogenase 2 promoter or metallothionine promoter. Suitable vectors include derivatives of the following types: self-replicating low copy number (such as the YRp-series), self-replicating high copy number (such as the YE<sub>p</sub>-series); integrating types (such as the YIp-series), or mini-chromosomes (such as the YC<sub>p</sub>-series).

Higher eukaryotic tissue culture cells are normally the preferred host cells for expression of the functionally active interleukin or receptor proteins. In principle, many higher eukaryotic tissue culture cell lines are workable, e.g., insect baculovirus expression systems, whether from an invertebrate or vertebrate source. However, mammalian cells are preferred. Transformation or transfection and propagation of such cells has become a routine procedure. Examples of useful cell lines include HeLa cells, Chinese hamster ovary (CHO) cell lines, baby rat kidney (BRK) cell lines, insect cell lines, bird cell lines, and monkey (COS) cell lines. Expression vectors for such cell lines usually include an origin of replication, a promoter, a translation initiation site, RNA splice sites (if genomic DNA is used), a polyadenylation site, and a transcription termination site. These vectors also usually contain a selection gene or amplification gene. Suitable expression vectors may be plasmids, viruses, or retroviruses carrying promoters derived, e.g., from such sources as from adenovirus, SV40, parvoviruses, vaccinia virus, or cytomegalovirus. Representative examples of suitable expression vectors include pCDNA1; pCD, see Okayama, et al. (1985) Mol. Cell Biol. 5:1136-1142; pMC1neo PolyA, see Thomas, et al. (1987) Cell 51:503-512; and a baculovirus vector such as pAC 373 or pAC 610.

For secreted proteins and some membrane proteins, an open reading frame usually encodes a polypeptide that consists of a mature or secreted product covalently linked at its N-terminus to a signal peptide. The signal peptide is cleaved prior to secretion of the mature, or active, polypeptide. The cleavage site can be predicted with a high degree of accuracy from empirical rules, e.g., von-Heijne (1986) Nucleic Acids Research 14:4683-4690; and Nielsen, et al. (1997) Protein Eng. 10:1-12, and the precise amino acid composition of the signal peptide often does not appear to be critical to its function, e.g.,

Randall, et al. (1989) Science 243:1156-1159; and Kaiser, et al. (1987) Science 235:312-317. The mature proteins of the invention can be readily determined using standard methods.

It will often be desired to express these polypeptides in a system which provides a specific or defined glycosylation pattern. In this case, the usual pattern will be that provided naturally by the expression system. However, the pattern will be modifiable by exposing the polypeptide, e.g., an unglycosylated form, to appropriate glycosylating proteins introduced into a heterologous expression system. For example, the receptor gene may be co-transformed with one or more genes encoding mammalian or other glycosylating enzymes. Using this approach, certain mammalian glycosylation patterns will be achievable in prokaryote or other cells. Expression in prokaryote cells will typically lead to unglycosylated forms of protein.

The source of DCRS8 can be a eukaryotic or prokaryotic host expressing recombinant DCRS8, such as is described above. The source can also be a cell line, but other mammalian cell lines are also contemplated by this invention, with the preferred cell line being from the human species.

Now that the sequences are known, the primate DCRS8 or DCRS9, fragments, or derivatives thereof can be prepared by conventional processes for synthesizing peptides. These include processes such as are described in Stewart and Young (1984) Solid Phase Peptide Synthesis, Pierce Chemical Co., Rockford, IL; Bodanszky and Bodanszky (1984) The Practice of Peptide Synthesis, Springer-Verlag, New York; and Bodanszky (1984) The Principles of Peptide Synthesis, Springer-Verlag, New York; all of each which are incorporated herein by reference. For example, an azide process, an acid chloride process, an acid anhydride process, a mixed anhydride process, an active ester process (for example, p-nitrophenyl ester, N-hydroxysuccinimide ester, or cyanomethyl ester), a carbodiimidazole process, an oxidative-reductive process, or a dicyclohexylcarbodiimide (DCCD)/additive process can be used. Solid phase and solution phase syntheses are both applicable to the foregoing processes. Similar techniques can be used with partial DCRS8 or DCRS9 sequences.

The DCRS8 proteins, fragments, or derivatives are suitably prepared in accordance with the above processes as typically employed in peptide synthesis, generally either by a so-called stepwise process which comprises condensing an amino acid to the terminal amino acid, one by one in sequence, or by coupling peptide fragments to the terminal amino acid. Amino groups that are not being used in the coupling reaction typically must be protected to prevent coupling at an incorrect location.

If a solid phase synthesis is adopted, the C-terminal amino acid is bound to an insoluble carrier or support through its carboxyl group. The insoluble carrier is not

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particularly limited as long as it has a binding capability to a reactive carboxyl group. Examples of such insoluble carriers include halomethyl resins, such as chloromethyl resin or bromomethyl resin, hydroxymethyl resins, phenol resins, tert-alkyloxycarbonylhydrazidated resins, and the like.

5 An amino group-protected amino acid is bound in sequence through condensation of its activated carboxyl group and the reactive amino group of the previously formed peptide or chain, to synthesize the peptide step by step. After synthesizing the complete sequence, the peptide is split off from the insoluble carrier to produce the peptide. This solid-phase approach is generally described by Merrifield, et al. (1963) in J. Am. Chem.  
10 Soc. 85:2149-2156, which is incorporated herein by reference.

The prepared protein and fragments thereof can be isolated and purified from the reaction mixture by means of peptide separation, e.g., by extraction, precipitation, electrophoresis, various forms of chromatography, and the like. The receptors of this invention can be obtained in varying degrees of purity depending upon desired uses.  
15 Purification can be accomplished by use of the protein purification techniques disclosed herein, see below, or by the use of the antibodies herein described in methods of immunoabsorbant affinity chromatography. This immunoabsorbant affinity chromatography is carried out by first linking the antibodies to a solid support and then contacting the linked antibodies with solubilized lysates of appropriate cells, lysates of  
20 other cells expressing the receptor, or lysates or supernatants of cells producing the protein as a result of DNA techniques, see below.

Generally, the purified protein will be at least about 40% pure, ordinarily at least about 50% pure, usually at least about 60% pure, typically at least about 70% pure, more typically at least about 80% pure, preferable at least about 90% pure and more preferably  
25 at least about 95% pure, and in particular embodiments, 97%-99% or more. Purity will usually be on a weight basis, but can also be on a molar basis. Different assays will be applied as appropriate. Individual proteins may be purified and thereafter combined.

## VI. Antibodies

30 Antibodies can be raised to the various mammalian, e.g., primate DCRS8 or DCRS9 proteins and fragments thereof, both in naturally occurring native forms and in their recombinant forms, the difference being that antibodies to the active receptor are more likely to recognize epitopes which are only present in the native conformations. Denatured antigen detection can also be useful in, e.g., Western analysis. Anti-idiotypic  
35 antibodies are also contemplated, which would be useful as agonists or antagonists of a natural receptor or an antibody.

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Antibodies, including binding fragments and single chain versions, against predetermined fragments of the protein can be raised by immunization of animals with conjugates of the fragments with immunogenic proteins. Monoclonal antibodies are prepared from cells secreting the desired antibody. These antibodies can be screened for binding to normal or defective protein, or screened for agonistic or antagonistic activity. These monoclonal antibodies will usually bind with at least a  $K_D$  of about 1 mM, more usually at least about 300  $\mu$ M, typically at least about 100 $\mu$ M, more typically at least about 30  $\mu$ M, preferably at least about 10  $\mu$ M, and more preferably at least about 3  $\mu$ M or better.

The antibodies, including antigen binding fragments, of this invention can have significant diagnostic or therapeutic value. They can be potent antagonists that bind to the receptor and inhibit binding to ligand or inhibit the ability of the receptor to elicit a biological response, e.g., act on its substrate. They also can be useful as non-neutralizing antibodies and can be coupled to toxins or radionuclides to bind producing cells, or cells localized to the source of the interleukin. Further, these antibodies can be conjugated to drugs or other therapeutic agents, either directly or indirectly by means of a linker.

The antibodies of this invention can also be useful in diagnostic applications. As capture or non-neutralizing antibodies, they might bind to the receptor without inhibiting ligand or substrate binding. As neutralizing antibodies, they can be useful in competitive binding assays. They will also be useful in detecting or quantifying ligand. They may be used as reagents for Western blot analysis, or for immunoprecipitation or immunopurification of the respective protein. Likewise, nucleic acids and proteins may be immobilized to solid substrates for affinity purification or detection methods. The substrates may be, e.g., solid resin beads or sheets of plastic.

Protein fragments may be joined to other materials, particularly polypeptides, as fused or covalently joined polypeptides to be used as immunogens. Mammalian cytokine receptors and fragments may be fused or covalently linked to a variety of immunogens, such as keyhole limpet hemocyanin, bovine serum albumin, tetanus toxoid, etc. See (1969) Microbiology, Hoeber Medical Division, Harper and Row; Landsteiner (1962) Specificity of Serological Reactions, Dover Publications, New York; and Williams, et al. (1967) Methods in Immunology and Immunochemistry, Vol. 1, Academic Press, New York; each of which is incorporated herein by reference, for descriptions of methods of preparing polyclonal antisera. A typical method involves hyperimmunization of an animal with an antigen. The blood of the animal is then collected shortly after the repeated immunizations and the gamma globulin is isolated.

In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts, such as mice, rodents, primates, humans, etc. Description of

techniques for preparing such monoclonal antibodies may be found in, e.g., Stites, et al. (eds.) Basic and Clinical Immunology (4th ed.), Lange Medical Publications, Los Altos, CA, and references cited therein; Harlow and Lane (1988) Antibodies: A Laboratory Manual, CSH Press; Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed.) Academic Press, New York; and particularly in Kohler and Milstein (1975) Nature 256:495-497, which discusses one method of generating monoclonal antibodies. Each of these references is incorporated herein by reference. Summarized briefly, this method involves injecting an animal with an immunogen. The animal is then sacrificed and cells taken from its spleen, which are then fused with myeloma cells. The result is a hybrid cell or "hybridoma" that is capable of reproducing in vitro. The population of hybridomas is then screened to isolate individual clones, each of which secrete a single antibody species to the immunogen. In this manner, the individual antibody species obtained are the products of immortalized and cloned single B cells from the immune animal generated in response to a specific site recognized on the immunogenic substance.

Other suitable techniques involve in vitro exposure of lymphocytes to the antigenic polypeptides or alternatively to selection of libraries of antibodies in phage or similar vectors. See, Huse, et al. (1989) "Generation of a Large Combinatorial Library of the Immunoglobulin Repertoire in Phage Lambda," Science 246:1275-1281; and Ward, et al. (1989) Nature 341:544-546, each of which is incorporated herein by reference. The polypeptides and antibodies of the present invention may be used with or without modification, including chimeric or humanized antibodies. Frequently, the polypeptides and antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like. Patents, teaching the use of such labels include U.S. Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also, recombinant or chimeric immunoglobulins may be produced, see Cabilly, U.S. Patent No. 4,816,567; or made in transgenic mice, see Mendez, et al. (1997) Nature Genetics 15:146-156; Abgenix; and Medarex. These references are incorporated herein by reference.

The antibodies of this invention can also be used for affinity chromatography in isolating the DCRS8 proteins or peptides. Columns can be prepared where the antibodies are linked to a solid support, e.g., particles, such as agarose, Sephadex, or the like, where a cell lysate may be passed through the column, the column washed, followed by increasing concentrations of a mild denaturant, whereby the purified protein will be

released. Alternatively, the protein may be used to purify antibody. Appropriate cross absorptions or depletions may be applied.

The antibodies may also be used to screen expression libraries for particular expression products. Usually the antibodies used in such a procedure will be labeled with a moiety allowing easy detection of presence of antigen by antibody binding.

Antibodies raised against a cytokine receptor will also be used to raise anti-idiotypic antibodies. These will be useful in detecting or diagnosing various immunological conditions related to expression of the protein or cells which express the protein. They also will be useful as agonists or antagonists of the ligand, which may be competitive inhibitors or substitutes for naturally occurring ligands.

INSA20 1a20 A cytokine receptor protein that specifically binds to or that is specifically immunoreactive with an antibody generated against a defined immunogen, such as an immunogen consisting of the amino acid sequence of SEQ ID NO: 14, is typically determined in an immunoassay. The immunoassay typically uses a polyclonal antiserum which was raised, e.g., to a protein of SEQ ID NO: 14. This antiserum is selected to have low crossreactivity against other cytokine receptor family members, preferably from the same species, and any such crossreactivity is removed by immunoabsorption prior to use in the immunoassay.

INSA21 1a21 In order to produce antisera for use in an immunoassay, the protein, e.g., of SEQ ID NO: 14, is isolated as described herein. For example, recombinant protein may be produced in a mammalian cell line. An appropriate host, e.g., an inbred strain of mice such as Balb/c, is immunized with the selected protein, typically using a standard adjuvant, such as Freund's adjuvant, and a standard mouse immunization protocol (see Harlow and Lane, supra). Alternatively, a synthetic peptide derived from the sequences disclosed herein and conjugated to a carrier protein can be used as an immunogen. Polyclonal sera are collected and titered against the immunogen protein in an immunoassay, e.g., a solid phase immunoassay with the immunogen immobilized on a solid support. Polyclonal antisera with a titer of  $10^4$  or greater are selected and tested for their cross reactivity against other cytokine receptor family members using a competitive binding immunoassay such as the one described in Harlow and Lane, supra, at pages 570-573. Preferably at least two cytokine receptor family members are used in this determination. These cytokine receptor family members can be produced as recombinant proteins and isolated using standard molecular biology and protein chemistry techniques as described herein.

INSA22 1a22 Immunoassays in the competitive binding format can be used for the crossreactivity determinations. For example, the protein of SEQ ID NO: 14 can be immobilized to a solid support. Proteins added to the assay compete with the binding of

~~the antisera to the immobilized antigen. The ability of the above proteins to compete with the binding of the antisera to the immobilized protein is compared to the other proteins.~~

The percent crossreactivity for the above proteins is calculated, using standard calculations. Those antisera with less than 10% crossreactivity with each of the proteins listed above are selected and pooled. The cross-reacting antibodies are then removed from the pooled antisera by immunoabsorption with the above-listed proteins.

~~The immunoabsorbed and pooled antisera are then used in a competitive binding immunoassay as described above to compare a second protein to the immunogen protein (e.g., the DCRS8 like protein of SEQ ID NO: 14). In order to make this comparison, the two proteins are each assayed at a wide range of concentrations and the amount of each protein required to inhibit 50% of the binding of the antisera to the immobilized protein is determined. If the amount of the second protein required is less than twice the amount of, the protein of the selected protein or proteins that is required, then the second protein is said to specifically bind to an antibody generated to the immunogen.~~

It is understood that these cytokine receptor proteins are members of a family of homologous proteins that comprise at least 9 so far identified members, 6 mammalian and 3 worm embodiments. For a particular gene product, such as the DCRS8, the term refers not only to the amino acid sequences disclosed herein, but also to other proteins that are allelic, non-allelic, or species variants. It is also understood that the terms include nonnatural mutations introduced by deliberate mutation using conventional recombinant technology such as single site mutation, or by excising short sections of DNA encoding the respective proteins, or by substituting new amino acids, or adding new amino acids. Such minor alterations typically will substantially maintain the immunoidentity of the original molecule and/or its biological activity. Thus, these alterations include proteins that are specifically immunoreactive with a designated naturally occurring DCRS8 protein. The biological properties of the altered proteins can be determined by expressing the protein in an appropriate cell line and measuring the appropriate effect, e.g., upon transfected lymphocytes. Particular protein modifications considered minor would include conservative substitution of amino acids with similar chemical properties, as described above for the cytokine receptor family as a whole. By aligning a protein optimally with the protein of the cytokine receptors and by using the conventional immunoassays described herein to determine immunoidentity, one can determine the protein compositions of the invention.

## VII. Kits and quantitation

Both naturally occurring and recombinant forms of the cytokine receptor like molecules of this invention are particularly useful in kits and assay methods. For

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example, these methods would also be applied to screening for binding activity, e.g., ligands for these proteins. Several methods of automating assays have been developed in recent years so as to permit screening of tens of thousands of compounds per year. See, e.g., a BIOMEK automated workstation, Beckman Instruments, Palo Alto, California, and Fodor, et al. (1991) Science 251:767-773, which is incorporated herein by reference. The latter describes means for testing binding by a plurality of defined polymers synthesized on a solid substrate. The development of suitable assays to screen for a ligand or agonist/antagonist homologous proteins can be greatly facilitated by the availability of large amounts of purified, soluble cytokine receptors in an active state such as is provided by this invention.

Purified protein can be coated directly onto plates for use in the aforementioned ligand screening techniques. However, non-neutralizing antibodies to these proteins can be used as capture antibodies to immobilize the respective receptor on the solid phase, useful, e.g., in diagnostic uses.

This invention also contemplates use of receptor subunit, fragments thereof, peptides, and their fusion products in a variety of diagnostic kits and methods for detecting the presence of the protein or its ligand. Alternatively, or additionally, antibodies against the molecules may be incorporated into the kits and methods. Typically the kit will have a compartment containing, e.g., a DCRS8 peptide or gene segment or a reagent which recognizes one or the other. Typically, recognition reagents, in the case of peptide, would be a receptor or antibody, or in the case of a gene segment, would usually be a hybridization probe.

A preferred kit for determining the concentration of DCRS8 in a sample would typically comprise a labeled compound, e.g., ligand or antibody, having known binding affinity for DCRS8, a source of DCRS8 (naturally occurring or recombinant) as a positive control, and a means for separating the bound from free labeled compound, e.g., a solid phase for immobilizing the DCRS8 in the test sample. Compartments containing reagents, and instructions, will normally be provided. Appropriate nucleic acid or protein containing kits are also provided.

Antibodies, including antigen binding fragments, specific for mammalian DCRS8 or a peptide fragment, or receptor fragments are useful in diagnostic applications to detect the presence of elevated levels of ligand and/or its fragments. Diagnostic assays may be homogeneous (without a separation step between free reagent and antibody-antigen complex) or heterogeneous (with a separation step). Various commercial assays exist, such as radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), enzyme-multiplied immunoassay technique (EMIT), substrate-labeled fluorescent immunoassay (SLFIA) and the like. For example, unlabeled

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antibodies can be employed by using a second antibody which is labeled and which recognizes the antibody to a cytokine receptor or to a particular fragment thereof. These assays have also been extensively discussed in the literature. See, e.g., Harlow and Lane (1988) Antibodies: A Laboratory Manual, CSH, and Coligan (ed. 1991 and periodic supplements) Current Protocols In Immunology Greene/Wiley, New York.

Anti-idiotypic antibodies may have similar use to serve as agonists or antagonists of cytokine receptors. These should be useful as therapeutic reagents under appropriate circumstances.

Frequently, the reagents for diagnostic assays are supplied in kits, so as to optimize the sensitivity of the assay. For the subject invention, depending upon the nature of the assay, the protocol, and the label, either labeled or unlabeled antibody, or labeled ligand is provided. This is usually in conjunction with other additives, such as buffers, stabilizers, materials necessary for signal production such as substrates for enzymes, and the like. Preferably, the kit will also contain instructions for proper use and disposal of the contents after use. Typically the kit has compartments for each useful reagent, and will contain instructions for proper use and disposal of reagents. Desirably, the reagents are provided as a dry lyophilized powder, where the reagents may be reconstituted in an aqueous medium having appropriate concentrations for performing the assay.

The aforementioned constituents of the diagnostic assays may be used without modification or may be modified in a variety of ways. For example, labeling may be achieved by covalently or non-covalently joining a moiety which directly or indirectly provides a detectable signal. In many of these assays, a test compound, cytokine receptor, or antibodies thereto can be labeled either directly or indirectly. Possibilities for direct labeling include label groups: radiolabels such as  $^{125}\text{I}$ , enzymes (U.S. Pat. No. 3,645,090) such as peroxidase and alkaline phosphatase, and fluorescent labels (U.S. Pat. No. 3,940,475) capable of monitoring the change in fluorescence intensity, wavelength shift, or fluorescence polarization. Both of the patents are incorporated herein by reference. Possibilities for indirect labeling include biotinylation of one constituent followed by binding to avidin coupled to one of the above label groups.

There are also numerous methods of separating the bound from the free ligand, or alternatively the bound from the free test compound. The cytokine receptor can be immobilized on various matrixes followed by washing. Suitable matrices include plastic such as an ELISA plate, filters, and beads. Methods of immobilizing the receptor to a matrix include, without limitation, direct adhesion to plastic, use of a capture antibody, chemical coupling, and biotin-avidin. The last step in this approach involves the precipitation of antibody/antigen complex by any of several methods including those

utilizing, e.g., an organic solvent such as polyethylene glycol or a salt such as ammonium sulfate. Other suitable separation techniques include, without limitation, the fluorescein antibody magnetizable particle method described in Rattle, et al. (1984) Clin. Chem. 30(9):1457-1461, and the double antibody magnetic particle separation as described in U.S. Pat. No. 4,659,678, each of which is incorporated herein by reference.

The methods for linking protein or fragments to various labels have been extensively reported in the literature and do not require detailed discussion here. Many of the techniques involve the use of activated carboxyl groups either through the use of carbodiimide or active esters to form peptide bonds, the formation of thioethers by reaction of a mercapto group with an activated halogen such as chloroacetyl, or an activated olefin such as maleimide, for linkage, or the like. Fusion proteins will also find use in these applications.

Another diagnostic aspect of this invention involves use of oligonucleotide or polynucleotide sequences taken from the sequence of an cytokine receptor. These sequences can be used as probes for detecting levels of the respective cytokine receptor in patients suspected of having an immunological disorder. The preparation of both RNA and DNA nucleotide sequences, the labeling of the sequences, and the preferred size of the sequences has received ample description and discussion in the literature. Normally an oligonucleotide probe should have at least about 14 nucleotides, usually at least about 18 nucleotides, and the polynucleotide probes may be up to several kilobases. Various labels may be employed, most commonly radionuclides, particularly  $^{32}\text{P}$ . However, other techniques may also be employed, such as using biotin modified nucleotides for introduction into a polynucleotide. The biotin then serves as the site for binding to avidin or antibodies, which may be labeled with a wide variety of labels, such as radionuclides, fluorescers, enzymes, or the like. Alternatively, antibodies may be employed which can recognize specific duplexes, including DNA duplexes, RNA duplexes, DNA-RNA hybrid duplexes, or DNA-protein duplexes. The antibodies in turn may be labeled and the assay carried out where the duplex is bound to a surface, so that upon the formation of duplex on the surface, the presence of antibody bound to the duplex can be detected. The use of probes to the novel RNA may be carried out in conventional techniques such as nucleic acid hybridization, plus and minus screening, recombinational probing, hybrid released translation (HRT), and hybrid arrested translation (HART). Antisense nucleic acids, which may be used to block protein expression, are also provided. See, e.g., Isis Pharmaceuticals, Sequitur, Inc., or Hybridon. This also includes amplification techniques such as polymerase chain reaction (PCR).

Diagnostic kits which also test for the qualitative or quantitative presence of other markers are also contemplated. Diagnosis or prognosis may depend on the combination



of multiple indications used as markers. Thus, kits may test for combinations of markers. See, e.g., Viallet, et al. (1989) Progress in Growth Factor Res. 1:89-97.

#### VIII. Therapeutic Utility

5 This invention provides reagents with significant therapeutic value. See, e.g., Levitzki (1996) Curr. Opin. Cell Biol. 8:239-244. The cytokine receptors (naturally occurring or recombinant), fragments thereof, mutein receptors, and antibodies, along with compounds identified as having binding affinity to the receptors or antibodies, should be useful in the treatment of conditions exhibiting abnormal expression of the  
10 receptors of their ligands. Such abnormality will typically be manifested by immunological disorders, e.g., innate immunity, or developmentally. Additionally, this invention should provide therapeutic value in various diseases or disorders associated with abnormal expression or abnormal triggering of response to the ligand. For example, the IL-1 ligands have been suggested to be involved in morphologic development, e.g.,  
15 dorso-ventral polarity determination, and immune responses, particularly the primitive innate responses. See, e.g., Sun, et al. (1991) Eur. J. Biochem. 196:247-254; and Hultmark (1994) Nature 367:116-117.

Recombinant cytokine receptors, muteins, agonist or antagonist antibodies thereto, or antibodies can be purified and then administered to a patient. These reagents can be  
20 combined for therapeutic use with additional active ingredients, e.g., in conventional pharmaceutically acceptable carriers or diluents, along with physiologically innocuous stabilizers and excipients. These combinations can be sterile, e.g., filtered, and placed into dosage forms as by lyophilization in dosage vials or storage in stabilized aqueous preparations. This invention also contemplates use of antibodies or binding fragments  
25 thereof which are not complement binding.

Ligand screening using cytokine receptor or fragments thereof can be performed to identify molecules having binding affinity to the receptors. Subsequent biological assays can then be utilized to determine if a putative ligand can provide competitive binding, which can block intrinsic stimulating activity. Receptor fragments can be used  
30 as a blocker or antagonist in that it blocks the activity of ligand. Likewise, a compound having intrinsic stimulating activity can activate the receptor and is thus an agonist in that it simulates the activity of ligand, e.g., inducing signaling. This invention further contemplates the therapeutic use of antibodies to cytokine receptors as antagonists.

The quantities of reagents necessary for effective therapy will depend upon many  
35 different factors, including means of administration, target site, reagent physiological life, pharmacological life, physiological state of the patient, and other medicants administered. Thus, treatment dosages should be titrated to optimize safety and efficacy. Typically,

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dosages used in vitro may provide useful guidance in the amounts useful for in situ administration of these reagents. Animal testing of effective doses for treatment of particular disorders will provide further predictive indication of human dosage. Various considerations are described, e.g., in Gilman, et al. (eds. 1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn.; each of which is hereby incorporated herein by reference. Methods for administration are discussed therein and below, e.g., for oral, intravenous, intraperitoneal, or intramuscular administration, transdermal diffusion, and others. Pharmaceutically acceptable carriers will include water, saline, buffers, and other compounds described, e.g., in the Merck Index, Merck & Co., Rahway, New Jersey. Because of the likely high affinity binding, or turnover numbers, between a putative ligand and its receptors, low dosages of these reagents would be initially expected to be effective. And the signaling pathway suggests extremely low amounts of ligand may have effect. Thus, dosage ranges would ordinarily be expected to be in amounts lower than 1 mM concentrations, typically less than about 10  $\mu$ M concentrations, usually less than about 100 nM, preferably less than about 10 pM (picomolar), and most preferably less than about 1 fM (femtomolar), with an appropriate carrier. Slow release formulations, or slow release apparatus will often be utilized for continuous administration.

Cytokine receptors, fragments thereof, and antibodies or its fragments, antagonists, and agonists, may be administered directly to the host to be treated or, depending on the size of the compounds, it may be desirable to conjugate them to carrier proteins such as ovalbumin or serum albumin prior to their administration. Therapeutic formulations may be administered in many conventional dosage formulations. While it is possible for the active ingredient to be administered alone, it is preferable to present it as a pharmaceutical formulation. Formulations comprise at least one active ingredient, as defined above, together with one or more acceptable carriers thereof. Each carrier must be both pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and not injurious to the patient. Formulations include those suitable for oral, rectal, nasal, or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by methods well known in the art of pharmacy. See, e.g., Gilman, et al. (eds. 1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn.; Avis, et al. (eds. 1993) Pharmaceutical Dosage Forms: Parenteral Medications Dekker, NY; Lieberman, et al. (eds. 1990) Pharmaceutical Dosage Forms: Tablets Dekker, NY; and

Lieberman, et al. (eds. 1990) Pharmaceutical Dosage Forms: Disperse Systems Dekker, NY. The therapy of this invention may be combined with or used in association with other therapeutic agents, particularly agonists or antagonists of other cytokine receptor family members.

#### IX. Screening

Drug screening using DCRS8 or fragments thereof can be performed to identify compounds having binding affinity to the receptor subunit, including isolation of associated components. Subsequent biological assays can then be utilized to determine if the compound has intrinsic stimulating activity and is therefore a blocker or antagonist in that it blocks the activity of the ligand. Likewise, a compound having intrinsic stimulating activity can activate the receptor and is thus an agonist in that it simulates the activity of a cytokine ligand. This invention further contemplates the therapeutic use of antibodies to the receptor as cytokine agonists or antagonists.

Similarly, complexes comprising multiple proteins may be used to screen for ligands or reagents capable of recognizing the complex. Most cytokine receptors comprise at least two subunits, which may be the same, or distinct. Alternatively, the transmembrane receptor may bind to a complex comprising a cytokine-like ligand associated with another soluble protein serving, e.g., as a second receptor subunit.

One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant DNA molecules expressing the DCRS8 in combination with another cytokine receptor subunit. Cells may be isolated which express a receptor in isolation from other functional receptors. Such cells, either in viable or fixed form, can be used for standard antibody/antigen or ligand/receptor binding assays. See also, Parce, et al. (1989) Science 246:243-247; and Owicki, et al. (1990) Proc. Nat'l Acad. Sci. USA 87:4007-4011, which describe sensitive methods to detect cellular responses. Competitive assays are particularly useful, where the cells (source of putative ligand) are contacted and incubated with a labeled receptor or antibody having known binding affinity to the ligand, such as  $^{125}\text{I}$ -antibody, and a test sample whose binding affinity to the binding composition is being measured. The bound and free labeled binding compositions are then separated to assess the degree of ligand binding. The amount of test compound bound is inversely proportional to the amount of labeled receptor binding to the known source. Many techniques can be used to separate bound from free ligand to assess the degree of ligand binding. This separation step could typically involve a procedure such as adhesion to filters followed by washing, adhesion to plastic followed by washing, or centrifugation of the cell membranes. Viable cells could also be used to screen for the effects of drugs on cytokine mediated functions, e.g., second messenger

levels, e.g.,  $\text{Ca}^{++}$ ; cell proliferation; inositol phosphate pool changes; and others. Some detection methods allow for elimination of a separation step, e.g., a proximity sensitive detection system. Calcium sensitive dyes will be useful for detecting  $\text{Ca}^{++}$  levels, with a fluorimeter or a fluorescence cell sorting apparatus.

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#### X. Ligands

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The descriptions of the DCRS8 herein provides means to identify ligands, as described above. Such ligand should bind specifically to the respective receptor with reasonably high affinity. Various constructs are made available which allow either labeling of the receptor to detect its ligand. For example, directly labeling cytokine receptor, fusing onto it markers for secondary labeling, e.g., FLAG or other epitope tags, etc., will allow detection of receptor. This can be histological, as an affinity method for biochemical purification, or labeling or selection in an expression cloning approach. A two-hybrid selection system may also be applied making appropriate constructs with the available cytokine receptor sequences. See, e.g., Fields and Song (1989) Nature 340:245-246.

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Most likely candidates will be structually related to members of the IL-17 family. See, e.g., USSN 09/480,287. - *abn*

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The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the inventions to the specific embodiments.

### EXAMPLES

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#### I. General Methods

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Some of the standard methods are described or referenced, e.g., in Maniatis, et al. (1982) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor Press; Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual, (2d ed.), vols. 1-3, CSH Press, NY; or Ausubel, et al. (1987 and Supplements) Current Protocols in Molecular Biology, Greene/Wiley, New York. Methods for protein purification include such methods as ammonium sulfate precipitation, column chromatography, electrophoresis, centrifugation, crystallization, and others. See, e.g., Ausubel, et al. (1987 and periodic supplements); Coligan, et al. (ed. 1996) and periodic supplements, Current Protocols In Protein Science Greene/Wiley, New York; Deutscher (1990) "Guide to Protein Purification" in Methods in Enzymology, vol. 182, and other volumes in this series; and manufacturer's literature on use of protein purification products, e.g., Pharmacia, Piscataway, N.J., or Bio-Rad, Richmond, CA. Combination

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with recombinant techniques allow fusion to appropriate segments, e.g., to a FLAG sequence or an equivalent which can be fused via a protease-removable sequence. See, e.g., Hochuli (1990) "Purification of Recombinant Proteins with Metal Chelate Absorbent" in Setlow (ed.) Genetic Engineering, Principle and Methods 12:87-98, Plenum Press, N.Y.; and Crowe, et al. (1992) OIAexpress: The High Level Expression & Protein Purification System QUIAGEN, Inc., Chatsworth, CA.

Computer sequence analysis is performed, e.g., using available software programs, including those from the GCG (U. Wisconsin) and GenBank sources. Public sequence databases were also used, e.g., from GenBank and others.

Many techniques applicable to IL-10 receptors may be applied to the DCRSs, as described, e.g., in USSN 08/110,683 (IL-10 receptor), which is incorporated herein by reference.

## II. Computational Analysis

Human sequences related to cytokine receptors were identified from genomic sequence database using, e.g., the BLAST server (Altschul, et al. (1994) Nature Genet. 6:119-129). Standard analysis programs may be used to evaluate structure, e.g., PHD (Rost and Sander (1994) Proteins 19:55-72) and DSC (King and Sternberg (1996) Protein Sci. 5:2298-2310). Standard comparison software includes, e.g., Altschul, et al. (1990) J. Mol. Biol. 215:403-10; Waterman (1995) Introduction to Computational Biology: Maps, Sequences, and Genomes Chapman & Hall; Lander and Waterman (eds. 1995) Calculating the Secrets of Life: Applications of the Mathematical Sciences in Molecular Biology National Academy Press; and Speed and Waterman (eds. 1996) Genetic Mapping and DNA Sequencing (IMA Volumes in Mathematics and Its Applications, Vol 81) Springer Verlag. Each reference is incorporate herein by reference.

## III. Cloning of full-length cDNAs; Chromosomal localization

~~PCR primers derived from the sequences are used to probe a human cDNA library. Sequences may be derived, e.g., from Tables 1-5, preferably those adjacent the ends of sequences. Full length cDNAs for primate, rodent, or other species DCRS8 are cloned, e.g., by DNA hybridization screening of  $\lambda$ gt10 phage. PCR reactions are conducted using T. aquaticus Taqplus DNA polymerase (Stratagene) under appropriate conditions. Extending partial length cDNA clones is typically routine.~~

Chromosome spreads are prepared. In situ hybridization is performed on chromosome preparations obtained from phytohemagglutinin-stimulated human lymphocytes cultured for 72 h. 5-bromodeoxyuridine was added for the final seven hours

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A PCR fragment, amplified with the help of primers, is cloned into an appropriate vector. The vector is labeled by nick-translation with  $^3\text{H}$ . The radiolabeled probe is hybridized to metaphase spreads at final concentration of 200 ng/ml of hybridization solution as described, e.g., in Mattei, et al. (1985) Hum. Genet. 69:327-331.

After coating with nuclear track emulsion (KODAK NTB2), slides are exposed. To avoid any slipping of silver grains during the banding procedure, chromosome spreads are first stained with buffered Giemsa solution and metaphase photographed. R-banding is then performed by the fluorochrome-photolysis-Giemsa (FPG) method and metaphases rephotographed before analysis.

Similar appropriate methods are used for other species.

#### IV. Localization of mRNA

Human multiple tissue (Cat# 1, 2) and cancer cell line blots (Cat# 7757-1), containing approximately 2  $\mu$ g of poly(A)<sup>+</sup> RNA per lane, are purchased from Clontech (Palo Alto, CA). Probes are radiolabeled with [ $\alpha$ -<sup>32</sup>P] dATP, e.g., using the Amersham Rediprime random primer labeling kit (RPN1633). Prehybridization and hybridizations are performed, e.g., at 65° C in 0.5 M Na<sub>2</sub>HPO<sub>4</sub>, 7% SDS, 0.5 M EDTA (pH 8.0). High stringency washes are conducted, e.g., at 65° C with two initial washes in 2 x SSC, 0.1% SDS for 40 min followed by a subsequent wash in 0.1 x SSC, 0.1% SDS for 20 min. Membranes are then exposed at -70° C to X-Ray film (Kodak) in the presence of intensifying screens. More detailed studies by cDNA library Southernblots are performed with selected appropriate human DCRS clones to examine their expression in hemopoietic or other cell subsets.

~~Alternatively, two appropriate primers are selected from Tables 1-5. RT-PCR is used on an appropriate mRNA sample selected for the presence of message to produce a cDNA, e.g., a sample which expresses the gene.~~

Full length clones may be isolated by hybridization of cDNA libraries from appropriate tissues pre-selected by PCR signal. Northern blots can be performed.

Message for genes encoding DCRS will be assayed by appropriate technology, e.g., PCR, immunoassay, hybridization, or otherwise. Tissue and organ cDNA preparations are available, e.g., from Clontech, Mountain View, CA. Identification of sources of natural expression are useful, as described. And the identification of functional receptor subunit pairings will allow for prediction of what cells express the combination of receptor subunits which will result in a physiological responsiveness to each of the cytokine ligands.

For mouse counterpart distribution, e.g., Southern Analysis can be performed: DNA (5 µg) from a primary amplified cDNA library was digested with appropriate restriction enzymes to release the inserts, run on a 1% agarose gel and transferred to a nylon membrane (Schleicher and Schuell, Keene, NH).

5 Samples for mouse mRNA isolation may include: resting mouse fibroblastic L cell line (C200); Braf:ER (Braf fusion to estrogen receptor) transfected cells, control (C201); T cells, TH1 polarized (Mel14 bright, CD4+ cells from spleen, polarized for 7 days with IFN-γ and anti IL-4; T200); T cells, TH2 polarized (Mel14 bright, CD4+ cells from spleen, polarized for 7 days with IL-4 and anti-IFN-γ; T201); T cells, highly TH1 polarized (see Openshaw, et al. (1995) J. Exp. Med. 182:1357-1367; activated with anti-CD3 for 2, 6, 16 h pooled; T202); T cells, highly TH2 polarized (see Openshaw, et al. (1995) J. Exp. Med. 182:1357-1367; activated with anti-CD3 for 2, 6, 16 h pooled; T203); CD44- CD25+ pre T cells, sorted from thymus (T204); TH1 T cell clone D1.1, resting for 3 weeks after last stimulation with antigen (T205); TH1 T cell clone D1.1, 10 µg/ml ConA stimulated 15 h (T206); TH2 T cell clone CDC35, resting for 3 weeks after last stimulation with antigen (T207); TH2 T cell clone CDC35, 10 µg/ml ConA stimulated 15 h (T208); Mel14+ naive T cells from spleen, resting (T209); Mel14+ T cells, polarized to Th1 with IFN-γ/IL-12/anti-IL-4 for 6, 12, 24 h pooled (T210); Mel14+ T cells, polarized to Th2 with IL-4/anti-IFN-γ for 6, 13, 24 h pooled (T211); unstimulated mature B cell leukemia cell line A20 (B200); unstimulated B cell line CH12 (B201); unstimulated large B cells from spleen (B202); B cells from total spleen, LPS activated (B203); metrizamide enriched dendritic cells from spleen, resting (D200); dendritic cells from bone marrow, resting (D201); monocyte cell line RAW 264.7 activated with LPS 4 h (M200); bone-marrow macrophages derived with GM and M-CSF (M201); macrophage cell line J774, resting (M202); macrophage cell line J774 + LPS + anti-IL-10 at 0.5, 1, 3, 6, 12 h pooled (M203); macrophage cell line J774 + LPS + IL-10 at 0.5, 1, 3, 5, 12 h pooled (M204); aerosol challenged mouse lung tissue, Th2 primers, aerosol OVA challenge 7, 14, 23 h pooled (see Garlisi, et al. (1995) Clinical Immunology and Immunopathology 75:75-83; X206); Nippostrongylus-infected lung tissue (see Coffman, et al. (1989) Science 245:308-310; X200); total adult lung, normal (O200); total lung, rag-1 (see Schwarz, et al. (1993) Immunodeficiency 4:249-252; O205); IL-10 K.O. spleen (see Kuhn, et al. (1991) Cell 75:263-274; X201); total adult spleen, normal (O201); total spleen, rag-1 (O207); IL-10 K.O. Peyer's patches (O202); total Peyer's patches, normal (O210); IL-10 K.O. mesenteric lymph nodes (X203); total mesenteric lymph nodes, normal (O211); IL-10 K.O. colon (X203); total colon, normal (O212); NOD mouse pancreas (see Makino, et al. (1980) Jikken Dobutsu 29:1-13; X205); total thymus, rag-1 (O208); total kidney, rag-1 (O209); total heart, rag-1 (O202); total brain, rag-1 (O203);

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total testes, rag-1 (O204); total liver, rag-1 (O206); rat normal joint tissue (O300); and rat arthritic joint tissue (X300).

Samples for human mRNA isolation may include, e.g.: peripheral blood mononuclear cells (monocytes, T cells, NK cells, granulocytes, B cells), resting (T100); peripheral blood mononuclear cells, activated with anti-CD3 for 2, 6, 12 h pooled (T101); T cell, TH0 clone Mot 72, resting (T102); T cell, TH0 clone Mot 72, activated with anti-CD28 and anti-CD3 for 3, 6, 12 h pooled (T103); T cell, TH0 clone Mot 72, anergic treated with specific peptide for 2, 7, 12 h pooled (T104); T cell, TH1 clone HY06, resting (T107); T cell, TH1 clone HY06, activated with anti-CD28 and anti-CD3 for 3, 6, 12 h pooled (T108); T cell, TH1 clone HY06, anergic treated with specific peptide for 2, 6, 12 h pooled (T109); T cell, TH2 clone HY935, resting (T110); T cell, TH2 clone HY935, activated with anti-CD28 and anti-CD3 for 2, 7, 12 h pooled (T111); T cells CD4+CD45RO- T cells polarized 27 days in anti-CD28, IL-4, and anti IFN- $\gamma$ , TH2 polarized, activated with anti-CD3 and anti-CD28 4 h (T116); T cell tumor lines Jurkat and Hut78, resting (T117); T cell clones, pooled AD130.2, Tc783.12, Tc783.13, Tc783.58, Tc782.69, resting (T118); T cell random  $\gamma\delta$  T cell clones, resting (T119); Splenocytes, resting (B100); Splenocytes, activated with anti-CD40 and IL-4 (B101); B cell EBV lines pooled WT49, RSB, JY, CVIR, 721.221, RM3, HSY, resting (B102); B cell line JY, activated with PMA and ionomycin for 1, 6 h pooled (B103); NK 20 clones pooled, resting (K100); NK 20 clones pooled, activated with PMA and ionomycin for 6 h (K101); NKL clone, derived from peripheral blood of LGL leukemia patient, IL-2 treated (K106); NK cytotoxic clone 640-A30-1, resting (K107); hematopoietic precursor line TF1, activated with PMA and ionomycin for 1, 6 h pooled (C100); U937 premonocytic line, resting (M100); U937 premonocytic line, activated with PMA and ionomycin for 1, 6 h pooled (M101); elutriated monocytes, activated with LPS, IFN $\gamma$ , anti-IL-10 for 1, 2, 6, 12, 24 h pooled (M102); elutriated monocytes, activated with LPS, IFN $\gamma$ , IL-10 for 1, 2, 6, 12, 24 h pooled (M103); elutriated monocytes, activated with LPS, IFN $\gamma$ , anti-IL-10 for 4, 16 h pooled (M106); elutriated monocytes, activated with LPS, IFN $\gamma$ , IL-10 for 4, 16 h pooled (M107); elutriated monocytes, activated LPS for 1 h (M108); elutriated monocytes, activated LPS for 6 h (M109); DC 70% CD1a+, from CD34+ GM-CSF, TNF $\alpha$  12 days, resting (D101); DC 70% CD1a+, from CD34+ GM-CSF, TNF $\alpha$  12 days, activated with PMA and ionomycin for 1 hr (D102); DC 70% CD1a+, from CD34+ GM-CSF, TNF $\alpha$  12 days, activated with PMA and ionomycin for 6 hr (D103); DC 95% CD1a+, from CD34+ GM-CSF, TNF $\alpha$  12 days FACS sorted, activated with PMA and ionomycin for 1, 6 h pooled (D104); DC 95% CD14+, ex CD34+ GM-CSF, TNF $\alpha$  12 days FACS sorted, activated with PMA and ionomycin 1, 6 hr pooled (D105); DC CD1a+ CD86+, from CD34+ GM-CSF, TNF $\alpha$  12 days FACS sorted, activated with PMA and

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ionomycin for 1, 6 h pooled (D106); DC from monocytes GM-CSF, IL-4 5 days, resting (D107); DC from monocytes GM-CSF, IL-4 5 days, resting (D108); DC from monocytes GM-CSF, IL-4 5 days, activated LPS 4, 16 h pooled (D109); DC from monocytes GM-CSF, IL-4 5 days, activated TNF $\alpha$ , monocyte supe for 4, 16 h pooled (D110); leiomyoma L11 benign tumor (X101); normal myometrium M5 (O115); malignant leiomyosarcoma GS1 (X103); lung fibroblast sarcoma line MRC5, activated with PMA and ionomycin for 1, 6 h pooled (C101); kidney epithelial carcinoma cell line CHA, activated with PMA and ionomycin for 1, 6 h pooled (C102); kidney fetal 28 wk male (O100); lung fetal 28 wk male (O101); liver fetal 28 wk male (O102); heart fetal 28 wk male (O103); brain fetal 28 wk male (O104); gallbladder fetal 28 wk male (O106); small intestine fetal 28 wk male (O107); adipose tissue fetal 28 wk male (O108); ovary fetal 25 wk female (O109); uterus fetal 25 wk female (O110); testes fetal 28 wk male (O111); spleen fetal 28 wk male (O112); adult placenta 28 wk (O113); and tonsil inflamed, from 12 year old (X100).

TaqMan quantitative PCR techniques have shown the DCRS6, in both mouse and human, to be expressed on T cells, including thymocytes and CD4+ naive and differentiated (hDCRS6 is also expressed on dendritic cells), in gastrointestinal tissue, including stomach, intestine, colon and associated lymphoid tissue, e.g., Peyer's patches and mesenteric lymph nodes, and upregulated in inflammatory models of bowel disease, e.g., IL-10 KO mice. The hDCRS7 was detected in both resting and activated dendritic cells, epithelial cells, and mucosal tissues, including GI and reproductive tracts. These data suggest that family members are expressed in mucosal tissues and immune system cell types, and/or in gastrointestinal, airway, and reproductive tract development.

As such, therapeutic indications include, e.g., short bowel syndrome, post chemo/radio-therapy or alcoholic recovery, combinations with ulcer treatments or arthritis medication, Th2 pregnancy skewing, stomach lining/tissue regeneration, loss of adsorptive surface conditions, etc. See, e.g., Yamada, et al. (eds. 1999) Textbook of Gastroenterology; Yamada, et al. (eds. 1999) Textbook and Atlas of Gastroenterology; Gore and Levine (2000) Textbook of Gastrointestinal Radiology; and (1987) Textbook of Pediatric Gastroenterology.

Similar samples may isolated in other species for evaluation.

~~Primers specific for IL-17RA were designed and used in Taqman quantative PCR against various human libraries. IL-17RA is highly expressed in innate immune myeloid cells including dendritic cells and monocytes. Expression is also detected in T-cell libraries. These data demonstrate the receptor is expressed in immune cell types and may be regulated by activation conditions.~~

1A27 ~~Table for IL-17RA~~  
~~Library description~~

CT for IL-  
 17RA\_H

DC ex monocytes GM-CSF, IL-4, resting	16.97
U937 premonocytic line, activated	17.14
DC ex monocytes GM-CSF, IL-4, resting	17.53
DC 70% CD1a+, ex CD34+ GM-CSF, TNFa, resting	18.17
monocytes, LPS, gIFN, anti-IL-10	18.27
DC ex monocytes GM-CSF, IL-4, LPS activated 4+16 hr	18.51
DC ex monocytes GM-CSF, IL-4, monokine activated 4+16 hr	18.68
kidney epithelial carcinoma cell line CHA, activated	18.69
monocytes, LPS, 1 hr	18.72
monocytes, LPS, 6 hr	18.72
DC 70% CD1a+, ex CD34+ GM-CSF, TNFa, activated 1 hr	18.91
DC 70% CD1a+, ex CD34+ GM-CSF, TNFa, activated 6 hr	18.94
T cell, TH1 clone HY06, activated	18.99
lung fetal	19.15
T cell, TH1 clone HY06, resting	19.18
T cell, TH1 clone HY06, anergic	19.23
monocytes, LPS, gIFN, IL-10, 4+16 hr	19.3
spleen fetal	19.51
testes fetal	19.7
T cell, TH0 clone Mot 72, resting	19.71
T cell, TH0 clone Mot 72, resting	19.84
DC CD1a+ CD86+, ex CD34+ GM-CSF, TNFa, activated 1+6 hr	19.94
peripheral blood mononuclear cells, activated	20.01
hematopoietic precursor line TF1, activated	20.07
lung fibroblast sarcoma line MRC5, activated	20.18
Splenocytes, activated	20.21
T cell gd clones, resting	20.27
ovary fetal	20.45
T cells CD4+, TH2 polarized, activated	20.57
Splenocytes, resting	20.6
uterus fetal	20.62
DC 95% CD1a+, ex CD34+ GM-CSF, TNFa, activated 1+6 hr	20.94
epithelial cells, unstimulated	20.96
peripheral blood mononuclear cells, resting	20.97
adipose tissue fetal	21.13

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B cell line JY, activated	21.28
monocytes, LPS, gIFN, IL-10	21.37
placenta 28 wk	21.38
NK 20 clones pooled, activated	21.55
pool of two normal human lung samples	21.63
normal human thyroid	21.65
epithelial cells, IL-1b activated	21.72
normal human skin	21.84
T cell, TH0 clone Mot 72, anergic	21.87
small intestine fetal	22.01
CD28- T cell clone in pME	22.08
T cell, TH2 clone HY935, activated	22.09
T cell clones, pooled, resting	22.29
Hashimoto's thyroiditis thyroid sample	22.3
NK 20 clones pooled, resting	22.4
B cell EBV lines, resting	22.45
T cell, TH2 clone HY935, resting	22.86
T cell, TH0 clone Mot 72, activated	23.3
monocytes, LPS, gIFN, anti-IL-10, 4+16 hr	23.39
T cell lines Jurkat and Hut78, resting	23.4
T cell, TH0 clone Mot 72, activated	23.56
<i>Pneumocystis carinii</i> pneumonia lung sample	24.05
U937 premonocytic line, resting	25.01
pool of rheumatoid arthritis samples, human	25.85
pool of three heavy smoker human lung samples	26.1
DC 95% CD14+, ex CD34+ GM-CSF, TNFa, activated 1+6 hr	32.69
kidney fetal	33.7
liver fetal	34.4
NK cytotoxic clone, resting	34.49
tonsil inflamed	35.02
normal w.t. monkey lung	35.45
gallbladder fetal	35.84
TR1 T cell clone	35.86
allergic lung sample	36.39
Psoriasis patient skin sample	36.44
normal human colon	37.34
brain fetal	37.35
<i>Ascaris</i> -challenged monkey lung, 4 hr.	37.75
<i>Ascaris</i> -challenged monkey lung, 24 hr.	40
heart fetal	40
normal w.t. monkey colon	40
ulcerative colitis human colon sample	40

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~~INSA28~~ 1028 Primers specific for DCRS6\_H were designed and used in Taqman quantitative PCR against various human libraries. DCRS6\_H is expressed in innate immune myeloid cells including dendritic cells and monocytes. Expression is also detected in T-cell libraries. These data demonstrate the receptor is expressed in immune cell types and may be regulated by activation conditions.

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~~INSA29~~ 1029 ~~Table for DCRS6\_H~~  
~~library description~~

	CT for DCRS6_H
T cell, TH0 clone Mot 72, resting	15.54
T cell, TH0 clone Mot 72, resting	15.7
DC ex monocytes GM-CSF, IL-4, resting	17.84
DC ex monocytes GM-CSF, IL-4, resting	18.19
DC ex monocytes GM-CSF, IL-4, LPS activated 4+16 hr	18.3
DC ex monocytes GM-CSF, IL-4, monokine activated 4+16 hr	18.3
T cell, TH1 clone HY06, resting	18.43
NK cytotoxic clone, resting	18.53
T cell clones, pooled, resting	18.8
T cell, TH1 clone HY06, activated	19.03
T cell, TH2 clone HY935, activated	19.1
TR1 T cell clone	19.12
T cells CD4+, TH2 polarized, activated	20.06
B cell EBV lines, resting	20.3
T cell, TH2 clone HY935, resting	20.48
kidney epithelial carcinoma cell line CHA, activated	21.07
T cell, TH1 clone HY06, anergic	21.14
normal human colon	21.29
NK 20 clones pooled, resting	21.49
T cell gd clones, resting	21.58
gallbladder fetal	22.21
kidney fetal	22.79
liver fetal	22.8
<i>Pneumocystis carinii</i> pneumonia lung sample	23.06
CD28- T cell clone in pME	23.18
T cell, TH0 clone Mot 72, anergic	23.2
ovary fetal	23.51
normal human thyroid	24.03
small intestine fetal	24.13
testes fetal	24.82
epithelial cells, IL-1b activated	26.08
pool of three heavy smoker human lung samples	26.49
placenta 28 wk	26.56
normal w.t. monkey lung	28.65
peripheral blood mononuclear cells,	33.39

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activated	
Ascaris-challenged monkey lung, 4 hr.	36.59
spleen fetal	38.43
peripheral blood mononuclear cells, resting	40
T cell, TH0 clone Mot 72, activated	40
T cell lines Jurkat and Hut78, resting	40
Splenocytes, resting	40
Splenocytes, activated	40
B cell line JY, activated	40
NK 20 clones pooled, activated	40
hematopoietic precursor line TF1, activated	40
U937 premonocytic line, resting	40
U937 premonocytic line, activated	40
monocytes, LPS, gIFN, anti-IL-10	40
monocytes, LPS, gIFN, IL-10	40
monocytes, LPS, gIFN, anti-IL-10, 4+16 hr	40
monocytes, LPS, gIFN, IL-10, 4+16 hr	40
monocytes, LPS, 1 hr	40
monocytes, LPS, 6 hr	40
DC 70% CD1a+, ex CD34+ GM-CSF, TNFa, resting	40
DC 70% CD1a+, ex CD34+ GM-CSF, TNFa, activated 1 hr	40
DC 70% CD1a+, ex CD34+ GM-CSF, TNFa, activated 6 hr	40
DC 95% CD1a+, ex CD34+ GM-CSF, TNFa, activated 1+6 hr	40
DC 95% CD14+, ex CD34+ GM-CSF, TNFa, activated 1+6 hr	40
DC CD1a+ CD86+, ex CD34+ GM-CSF, TNFa, activated 1+6 hr	40
epithelial cells, unstimulated	40
lung fibroblast sarcoma line MRC5, activated	40
Ascaris-challenged monkey lung, 24 hr.	40
pool of two normal human lung samples	40
allergic lung sample	40
normal w.t. monkey colon	40
ulcerative colitis human colon sample	40
Hashimoto's thyroiditis thyroid sample	40
pool of rheumatoid arthritis samples, human	40
normal human skin	40
Psoriasis patient skin sample	40
tonsil inflammed	40
lung fetal	40
heart fetal	40
brain fetal	40
adipose tissue fetal	40
uterus fetal	40

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T cell, TH0 clone Mot 72, activated

40

~~Primers specific for DCRS7\_H were designed and used in Taqman quantitative PCR against various human libraries. DCRS7\_H is expressed in innate immune myeloid cells including dendritic cells and monocytes. Expression is also detected in fetal libraries. These data demonstrate the receptor is expressed in immune cell types and may be regulated by activation conditions.~~

~~Table for DCRS7\_H-  
library description~~

CT for  
DCRS7\_H

fetal uterus	19.05
DC mix	19.34
fetal small intestine	19.46
fetal ovary	19.68
fetal testes	19.75
fetal lung	20.04
CHA	20.24
normal thyroid	20.32
DC/GM/IL-4	20.52
fetal spleen	20.86
normal lung	20.94
TF1	21
allergic lung #19	21.02
Psoriasis skin	21.07
fetal liver	21.15
MRC5	21.15
24 hr. Ascaris lung	21.17
hi dose IL-4 lung	21.23
CD1a+ 95%	21.32
Hashimotos thyroiditis	21.35
Crohns colon 4003197A	21.35
normal lung pool	21.36
70% DC resting	21.42
fetal kidney	21.58
adult placenta	21.68
lung 121897-1	21.8
Pneumocystis carinii lung #20	21.81
A549 unstim.	21.89
normal colon #22	21.94
18 hr. Ascaris lung	22.09
normal skin	22.1
Crohns colon 9609C144	22.13
fetal adipose tissue	22.35
D6	22.39

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DC resting CD34-derived	22.45
DC TNF/TGFb act CD34-der.	22.54
fetal brain	22.9
DC CD40L activ. mono-	22.91
deriv.	
Crohns colon 403242A	22.91
ulcerative colitis colon	23
#26	
RA synovium pool	23.06
A549 activated	23.06
mono + IL-10	23.42
DC LPS	23.49
Mot 72 activated	23.66
CD1a+ CD86+	23.86
HY06 resting	23.87
U937 activated	23.97
inflamed tonsil	23.97
D1	24.06
M1	24.17
CD14+ 95%	24.21
lung 080698-2	24.28
4 hr. Ascaris lung	24.37
Jurkat activated pSPORT	24.42
DC resting mono-derived	24.48
HY06 activated	24.54
C+	24.64
Splenocytes resting	24.65
U937/CD004 resting	24.96
PBMC resting	25.8
Mot 72 resting	25.91
mono + anti-IL-10	26.14
NK pool	26.99
HY06 anti-peptide	27.34
mast cell pME	27.38
Tc gamma delta	28.14
TC1080 CD28- pMET7	31.05
PBMC activated	31.89
NK non cytotox.	32.3
RV-C30 TR1 pMET7	32.5
Bc	33.72
C-	33.8
Splenocytes activated	34.7
JY	35.05
NK cytotox.	36.44
NKL/IL-2	37.59
HY935 resting	37.6
NK pool activated	38.15
Mot 72 anti-peptide	38.87
fetal heart	40.92

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B21 resting	42.05
Jurkat resting pSPORT	42.8
B21 activated	43.09
NKA6 pSPORT	44.85
HY935 activated	45
M6	45

~~1a32~~ ~~Primers specific for DCRS9\_H were designed and used in Taqman quantitative PCR against various human libraries. DCRS9\_H is expressed T-cells, fetal lung, and resting monocytes. These data demonstrate the receptor is expressed in immune cell types and may be regulated by activation conditions.~~

~~1a33~~ ~~Table for DCRS9\_H library description CT for~~

<del>DCRS9_H</del>	
HY06 resting	22.35
fetal lung	22.63
HY06 anti-peptide	22.72
HY06 activated	22.96
U937/CD004 resting	24.16
fetal small intestine	24.94
JY	25.04
Mot 72 resting	25.12
Jurkat activated pSPORT	25.2
RV-C30 TR1 pMET7	26.51
fetal kidney	26.76
MRC5	27.2
Psoriasis skin	27.3
Tc gamma delta	27.37
Crohns colon 4003197A	27.44
fetal spleen	27.72
normal lung	27.83
Hashimotos thyroiditis	28.03
B21 resting	28.32
TF1	28.39
NK cytotox.	28.44
TC1080 CD28- pMET7	28.61
Pneumocystis carinii lung #20	29.05
U937 activated	29.06
HY935 resting	29.09
CD1a+ 95%	29.13



B21 activated	29.2
Mot 72 activated	29.21
fetal testes	29.27
lung 080698-2	29.32
Jurkat resting	29.38
pSPORT	
CD14+ 95%	29.38
normal thyroid	29.53
Mot 72 anti-peptide	29.65
Splenocytes resting	29.85
Crohns colon 9609C144	30.28
lung 121897-1	30.37
24 hr. Ascaris lung	30.59
hi dose IL-4 lung	30.8
CD1a+ CD86+	31.42
normal skin	31.73
fetal uterus	31.79
PBMC activated	31.82
inflammed tonsil	31.98
fetal brain	32.21
RA synovium pool	32.77
allergic lung #19	33.18
18 hr. Ascaris lung	33.42
adult placenta	33.43
normal lung pool	33.45
Crohns colon 403242A	33.52
NK pool	33.72
HY935 activated	33.75
DC/GM/IL-4	34.28
DC resting mono-derived	34.57
fetal ovary	35.06
fetal adipose tissue	35.07
CHA	35.2
PBMC resting	35.95
Bc	36.19
A549 unstim.	36.4
fetal heart	36.87
ulcerative colitis colon #26	37.83
C-	38.32
4 hr. Ascaris lung	40.2
D6	40.62
C+	44.38

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A549 activated	44.58
Splenocytes	45
activated	
NK pool activated	45
NKA6 pSPORT	45
NKL/IL-2	45
NK non cytotox.	45
mono + anti-IL-10	45
mono + IL-10	45
M1	45
M6	45
70% DC resting	45
D1	45
DC LPS	45
DC mix	45
fetal liver	45
mast cell pME	45
DC CD40L activ.	45
mono-deriv.	
DC resting CD34-	45
derived	
DC TNF/TGFb act	45
CD34-der.	
normal colon #22	45

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## V. Cloning of species counterparts

Various strategies are used to obtain species counterparts of the DCRSs, preferably from other primates or rodents. One method is by cross hybridization using closely related species DNA probes. It may be useful to go into evolutionarily similar species as intermediate steps. Another method is by using specific PCR primers based on the identification of blocks of similarity or difference between genes, e.g., areas of highly conserved or nonconserved polypeptide or nucleotide sequence. Sequence database searches may identify species counterparts.

## VI. Production of mammalian protein

An appropriate, e.g., GST, fusion construct is engineered for expression, e.g., in *E. coli*. For example, a mouse IGIF pGex plasmid is constructed and transformed into *E. coli*. Freshly transformed cells are grown, e.g., in LB medium containing 50 µg/ml ampicillin and induced with IPTG (Sigma, St. Louis, MO). After overnight induction, the bacteria are harvested and the pellets containing the appropriate protein are isolated. The pellets are homogenized, e.g., in TE buffer (50 mM Tris-base pH 8.0, 10 mM EDTA and 2 mM pefabloc) in 2 liters. This material is passed through a microfluidizer (Microfluidics, Newton, MA) three times. The fluidized supernatant is spun down on a Sorvall GS-3 rotor for 1 h at 13,000 rpm. The resulting supernatant containing the cytokine receptor protein is filtered and passed over a glutathione-SEPHAROSE column equilibrated in 50 mM Tris-base pH 8.0. Fractions containing the DCRS8-GST fusion protein are pooled and cleaved, e.g., with thrombin (Enzyme Research Laboratories, Inc., South Bend, IN). The cleaved pool is then passed over a Q-SEPHAROSE column equilibrated in 50 mM Tris-base. Fractions containing DCRS8 are pooled and diluted in cold distilled H<sub>2</sub>O, to lower the conductivity, and passed back over a fresh Q-Sepharose column, alone or in succession with an immunoaffinity antibody column. Fractions containing the DCRS8 protein are pooled, aliquoted, and stored in the -70° C freezer.

Comparison of the CD spectrum with cytokine receptor protein may suggest that the protein is correctly folded. See Hazuda, et al. (1969) *J. Biol. Chem.* 264:1689-1693.

## VII. Preparation of specific antibodies

Inbred Balb/c mice are immunized intraperitoneally with recombinant forms of the protein, e.g., purified DCRS8 or stable transfected NIH-3T3 cells. Animals are boosted at appropriate time points with protein, with or without additional adjuvant, to further stimulate antibody production. Serum is collected, or hybridomas produced with harvested spleens.

Alternatively, Balb/c mice are immunized with cells transformed with the gene or fragments thereof, either endogenous or exogenous cells, or with isolated membranes enriched for expression of the antigen. Serum is collected at the appropriate time, typically after numerous further administrations. Various gene therapy techniques may be useful, e.g., in producing protein in situ, for generating an immune response. Serum or antibody preparations may be cross-absorbed or immunoselected to prepare substantially purified antibodies of defined specificity and high affinity.

Monoclonal antibodies may be made. For example, splenocytes are fused with an appropriate fusion partner and hybridomas are selected in growth medium by standard procedures. Hybridoma supernatants are screened for the presence of antibodies which bind to the DCRS8, e.g., by ELISA or other assay. Antibodies which specifically recognize specific DCRS8 embodiments may also be selected or prepared.

In another method, synthetic peptides or purified protein are presented to an immune system to generate monoclonal or polyclonal antibodies. See, e.g., Coligan (ed. 1991) Current Protocols in Immunology Wiley/Greene; and Harlow and Lane (1989) Antibodies: A Laboratory Manual Cold Spring Harbor Press. In appropriate situations, the binding reagent is either labeled as described above, e.g., fluorescence or otherwise, or immobilized to a substrate for panning methods. Nucleic acids may also be introduced into cells in an animal to produce the antigen, which serves to elicit an immune response. See, e.g., Wang, et al. (1993) Proc. Nat'l. Acad. Sci. 90:4156-4160; Barry, et al. (1994) BioTechniques 16:616-619; and Xiang, et al. (1995) Immunity 2: 129-135.

#### VIII. Production of fusion proteins

Various fusion constructs are made with DCRS8 or DCRS9. A portion of the appropriate gene is fused to an epitope tag, e.g., a FLAG tag, or to a two hybrid system construct. See, e.g., Fields and Song (1989) Nature 340:245-246.

The epitope tag may be used in an expression cloning procedure with detection with anti-FLAG antibodies to detect a binding partner, e.g., ligand for the respective cytokine receptor. The two hybrid system may also be used to isolate proteins which specifically bind to the receptor subunit.

#### IX. Structure activity relationship

Information on the criticality of particular residues is determined using standard procedures and analysis. Standard mutagenesis analysis is performed, e.g., by generating many different variants at determined positions, e.g., at the positions identified above, and evaluating biological activities of the variants. This may be performed to the extent of determining positions which modify activity, or to focus on specific positions to

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determine the residues which can be substituted to either retain, block, or modulate biological activity.

Alternatively, analysis of natural variants can indicate what positions tolerate natural mutations. This may result from populational analysis of variation among individuals, or across strains or species. Samples from selected individuals are analyzed, e.g., by PCR analysis and sequencing. This allows evaluation of population polymorphisms.

#### X. Isolation of a ligand

A cytokine receptor can be used as a specific binding reagent to identify its binding partner, by taking advantage of its specificity of binding, much like an antibody would be used. The binding receptor may be a heterodimer of receptor subunits; or may involve, e.g., a complex of the DCRS8 with another cytokine receptor subunit. A binding reagent is either labeled as described above, e.g., fluorescence or otherwise, or immobilized to a substrate for panning methods.

The binding composition is used to screen an expression library made from a cell line which expresses a binding partner, i.e., ligand, preferably membrane associated. Standard staining techniques are used to detect or sort surface expressed ligand, or surface expressing transformed cells are screened by panning. Screening of intracellular expression is performed by various staining or immunofluorescence procedures. See also McMahan, et al. (1991) *EMBO J.* 10:2821-2832.

For example, on day 0, precoat 2-chamber permanox slides with 1 ml per chamber of fibronectin, 10 ng/ml in PBS, for 30 min at room temperature. Rinse once with PBS. Then plate COS cells at  $2-3 \times 10^5$  cells per chamber in 1.5 ml of growth media. Incubate overnight at 37 C.

On day 1 for each sample, prepare 0.5 ml of a solution of 66  $\mu$ g/ml DEAE-dextran, 66  $\mu$ M chloroquine, and 4  $\mu$ g DNA in serum free DME. For each set, a positive control is prepared, e.g., of DCRS8-FLAG cDNA at 1 and 1/200 dilution, and a negative mock. Rinse cells with serum free DME. Add the DNA solution and incubate 5 hr at 37 C. Remove the medium and add 0.5 ml 10% DMSO in DME for 2.5 min. Remove and wash once with DME. Add 1.5 ml growth medium and incubate overnight.

On day 2, change the medium. On days 3 or 4, the cells are fixed and stained. Rinse the cells twice with Hank's Buffered Saline Solution (HBSS) and fix in 4% paraformaldehyde (PFA)/glucose for 5 min. Wash 3X with HBSS. The slides may be stored at -80 C after all liquid is removed. For each chamber, 0.5 ml incubations are performed as follows. Add HBSS/saponin (0.1%) with 32  $\mu$ l/ml of 1 M  $\text{NaN}_3$  for 20 min. Cells are then washed with HBSS/saponin 1X. Add appropriate DCRS8 or

DCRS8/antibody complex to cells and incubate for 30 min. Wash cells twice with HBSS/saponin. If appropriate, add first antibody for 30 min. Add second antibody, e.g., Vector anti-mouse antibody, at 1/200 dilution, and incubate for 30 min. Prepare ELISA solution, e.g., Vector Elite ABC horseradish peroxidase solution, and preincubate for 30 min. Use, e.g., 1 drop of solution A (avidin) and 1 drop solution B (biotin) per 2.5 ml HBSS/saponin. Wash cells twice with HBSS/saponin. Add ABC HRP solution and incubate for 30 min. Wash cells twice with HBSS, second wash for 2 min, which closes cells. Then add Vector diaminobenzoic acid (DAB) for 5 to 10 min. Use 2 drops of buffer plus 4 drops DAB plus 2 drops of H<sub>2</sub>O<sub>2</sub> per 5 ml of glass distilled water.

Carefully remove chamber and rinse slide in water. Air dry for a few minutes, then add 1 drop of Crystal Mount and a cover slip. Bake for 5 min at 85-90 C.

Evaluate positive staining of pools and progressively subclone to isolation of single genes responsible for the binding.

Alternatively, receptor reagents are used to affinity purify or sort out cells expressing a putative ligand. See, e.g., Sambrook, et al. or Ausubel, et al.

Another strategy is to screen for a membrane bound receptor by panning. The receptor cDNA is constructed as described above. Immobilization may be achieved by use of appropriate antibodies which recognize, e.g., a FLAG sequence of a DCRS8 fusion construct, or by use of antibodies raised against the first antibodies. Recursive cycles of selection and amplification lead to enrichment of appropriate clones and eventual isolation of receptor expressing clones.

Phage expression libraries can be screened by mammalian DCRS8. Appropriate label techniques, e.g., anti-FLAG antibodies, will allow specific labeling of appropriate clones.

We tested the ability of DCRS receptors to specifically bind IL-17 family cytokines. Recombinant FLAG-hIL-17 family cytokines were used in binding experiments on Baf/3 DCRS receptor transfected expressing recombinant IL-17R\_H, DCRS6\_H, DCRS7\_H, DCRS8\_H and DCRS9\_H and analyzed by FACS. We can demonstrate specific binding of IL-17 family member IL-74 to DCRS6 expressing Baf/3 cells. In additional experiments we have shown IL-17 specific binding to IL-17R\_H, DCRS7\_H, DCRS8\_H. Further experiments show IL-71 binding to DCRS8\_Hu transfectants. These experiments demonstrate the sequence homology among IL-17 related cytokine receptors confers functional binding to IL-17 cytokines.

All citations herein are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

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Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled; and the invention is not to be limited by the specific embodiments that have been presented herein by way of example.

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